FACTORS REGULATING BLOOD PRESSURE

Transactions of the Fifth Conference

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Edited by

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JOSIAH MACY, JR FOUNDATION CONFERENCE PROGRAM

FRANK FREMONT SMITH
Medical Director

I WANT TO welcome you and tell you how happy we are to have you all here at this fifth and last Conference on Factors Regulating Blood Pressure For the benefit of the guests who are present for the first time I should like to outline briefly the organization of these conferences and what we hope to accomplish through the Conference Program

We feel that nature has been somewhat artificially fragmented by our university departments and by our specialization into disciplines for if we are to understand fully any one part of nature we must see that part in relation to the whole Such perspective is extremely difficult if not impossible when we limit our view to what we can see through the lens of a single discipline We conceived these conferences therefore as an attempt to provide a planned opportunity not ordinarily available for multiprofessional communication around a given topic Although the fertility of the multidiscipline approach is recognized in principle universities scientific societies and journals have not yet made adequate provision for channels of interdisciplinary communication

In addition to this Conference we now have twelve similar groups covering a wide variety of topics Two new conferences have been organized one on cold injury the first meeting of which was held in June 1951 and one on shock and vascular homeostasis which will hold its first session in October 1951

There is usually a nucleus of members who come regularly every year and in addition there are guests who are invited for one or more meetings for their special contributions The setup of the meeting is not for the presentation of formal papers but for informal and free discussion The main purpose of the gathering is for the interchange of ideas the back and forth discussion between those present for mutual stimulation and for communication between the disciplines I am sure you are all to some extent aware

of the difficulty of communication between disciplines, even such *disciplines as anatomy and physiology*

When you begin to deal with a problem — and we have in this area such a problem — which crosses all the way from the physical and biological sciences to the psychological and social sciences then you are faced with the very great difficulty of communication between physical and biological sciences and social sciences

In a sense, we feel that medicine is in the key position to promote better communication Today medicine must be well versed in nuclear physics because of the tracer techniques and the injury that can come from radiation, for example

In addition medicine is a social science and through mental health must be concerned with public health problems of social and economic importance In the concept of psychosomatic medicine where we see psychological and social factors changing the functions of organs and organ systems and enzyme activity of cells we begin to see the absolute necessity for understanding and communication between the social scientist and the enzyme chemist At the present time we really have nature pretty much fragmented by our *disciplines which are set up in the universities by departments with* walls between them

One of the other things we discovered was that it was very much easier for a representative of one discipline to communicate with a representative of quite a different discipline if they came from different universities You might think that over also but it is really true And so we found it would be a great advantage to bring them together from different universities from different disciplines Obviously with twenty five as a maximum that we can bring to a meeting we must leave out a vast majority of the best people in every field if we have seven or eight disciplines represented by three or four people — and in any important field there are going to be twenty five to seventy five key people — we must face the fact that we cannot include everybody The smaller the group, the better the communication We have played back and forth with this process and have found that a group of twenty five people for a two day period is the optimum

I always like my chance to get in these introductory remarks which must echo and re echo in the ears of the members because they have heard them said so often You see a stenotypist here

taking down everything you say Well that is not as bad as it seems, because you will get a transcript of the complete Conference and you will have the opportunity to cross out anything that you wish you hadn t said or at least don t want to have in print Therefore we want to encourage you to speak with absolute freedom complete informality and not be the least inhibited Don t do what one man did – he crossed out everything he said We prefer not to have that happen We also would like to say don t hesitate to ask seemingly foolish questions because how can we be sure they won t evoke wisdom in somebody else? It is the evocation of wisdom rather than the statements we make with which we are concerned

This then is an experiment in communication Each of our conferences has an immediate purpose – to advance research in a particular area We like you to feel that you are a part of that experiment that you contribute to it not only by your efforts at communication but also by giving us some wisdom Will you send us your comments afterwards as to what can be done to better this communicative process? We want to share what we are doing here as much as possible through the transactions You will have in front of you the volume from the last meeting and you will see that a good deal of the informality at that meeting has been preserved in the publication

Not only are scientific meetings somewhat stereotyped but, far worse scientific publications are very seriously stereotyped they are in an editorial strait jacket I hope if there are any editors here they will bear with me and be somewhat tolerant of what I am saying but I do not think there is any question but that there is perversion of the truth in the majority of our scientific publications I mean that we have presented to the public and to each other only the logical rearrangement of our research work Research is not always logical. It has a logical end it always has but most important its essence is the creative part The creative is editorially censored and we are forced to rearrange what we actually do in the laboratory and put it into a logical sequence

In research what frequently happens is that you begin with an hypothesis which may or may not be correct You test it perhaps you get a hunch or lead from someone else you may make two or three false starts before your research gets under way Consequently the final results may have little connection with the original idea

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INTRODUCTORY REMARKS

HARRY GOLDBLATT
Chairman

ALL OF you who are familiar with what I have written in the last few years know that almost invariably I have concluded with the statement that in my opinion problem number one still remaining to be solved deals with the etiology and pathogenesis of the arterial and arteriolar sclerosis that usually accompanies hypertension I still believe that

Dr Fremont Smith has just told us that this is the fifth and last Conference on Factors Regulating Blood Pressure under my chairmanship It seemed appropriate therefore to end my period of conducting these conferences with a session on the subject I have mentioned

I think that if any one area of agreement has come out of these conferences about the origin of hypertension it is that it does appear to be due mainly to increased peripheral vascular resistance But whether the increased peripheral vascular resistance is always on a purely functional basis at least in the beginning whether it is both on a functional and an organic basis or only the latter is not established Finally we have not yet elucidated either the etiology or the pathogenesis of the athero or arteriosclerosis of the large and small arteries which could be the organic basis of hypertension What has given the greatest impetus to the investigation of this part of the problem is the recent work on the lipids in the blood and in the sites where they are deposited This therefore seems to be the opportune time in which to thrash out this subject As Dr Fremont Smith told you our primary purpose is to get provocative statements from the various participants which we hope will evoke much discussion from all those who are familiar with the subject or who have an idea or thought about it

We use logic to obtain our results, but if we present only the logical end, we deprive our audience of sharing in the interesting and exciting aspects of our research experience There is a further and potentially more serious consideration, namely the tendency on the part of nonscientific administrators to feel that science being logical, can be managed that it can be controlled that it can be ordered No one in a free country would think of ordering an artist to paint a landscape in a particular way I think the artistic aspects of science are as vital as the logical aspects We feel that in a small way the give and take in these informal discussions preserved in these transactions will give both sides of the *process of scientific thought* Therefore we hope that you will talk about your headaches your difficulties, and that you will even have *hunches* and express them in these meetings

The transactions are secondary to the discussions They are the tail and they must not wag the dog The dog is what actually happens here among you

We think that Dale Carnegie really had something in the title of his book *How to Win Friends and Influence People* We feel you will be able to communicate with each other only when you have established a relationship of confidence, a friendly relationship Therefore as part of the process of communication we want you to have the chance to associate with one another closely for the two day period We have seen peoples work modified, we have seen spontaneous collaboration take place between different universities between members of a conference collaboration not ordered by anyone from above but coming out of a mutual need to approach a problem from several different angles simultaneously

CHEMICAL STUDIES OF THE BLOOD IN ARTERIOSCLEROSIS

DAVID P BARR

Department of Internal Medicine
Cornell University Medical College

The observations that I shall report have been made in collaboration with Miss Ella Russ chemist of the Central Laboratories at New York Hospital and Dr Howard Eder of the Department of Medicine of Cornell

It has long been suspected that deposits of lipids in tissues are dependent upon the concentration of cholesterol and other lipid substances in the circulating blood In the past however too little account has been taken of the complexities of lipid relationships Evidence is now convincing that cholesterol and phospholipids do not exist in a free state in the plasma but under ordinary circumstances are combined with each other and with proteins in large molecules two groups of which the alphalipoproteins and the betalipoproteins have been extensively studied and partially characterized

It is conceivable that from the standpoint of the pathogenesis of atherosclerosis consideration of the concentration and distribution of lipoproteins may be more rewarding than that of any one of their constituents The possibility has been emphasized by the demonstration in Cohn s laboratory that all of the cholesterol of the plasma is distributed between two protein fractions and that in pooled plasma approximately 25 percent of the total cholesterol is in the alphalipoproteins the remainder forming part of the betalipoproteins Furthermore the recently published Method 10 of Cohn(1) for microfractionation of proteins makes possible effective separation of alpha and betalipoproteins in samples of blood sufficiently small for withdrawal from an individual

The primary object of the present study was to explore the possibility of significant fluctuation in the distribution of lipoproteins in health and disease Focus upon atherosclerosis diabetes and nephritis was not originally intended but was sharpened when we encountered quite early apparently significant variations in the

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plasma of patients suffering from the serious complications of atherosclerosis

For purposes of the investigation we used Cohn's Method 10 with no essential modifications As is well known this method employs a system of extreme ingenuity in which pH alcohol concentration, ionic strength protein concentration and temperature are critically controlled and in which the dipolar ion, glycine is used in high concentration to separate combinations of proteins

TABLE I

Cohn Fractionation—Method 10

FRACTION A(IV V VI)
- Albumin
- *Alpha$_1$ lipoprotein*
- Alpha glycoprotein
- Beta$_1$ metal combining protein

FRACTION B(II)
- Gamma globulins

FRACTION C(III$_0$)
- *Beta$_1$ lipoprotein*
- Beta$_1$ lipid poor euglobulin
- Ceruloplasmin

FRACTION D(I III)
- Fibrinogen
- Prothrombin
- Cold insoluble globulin
- Plasminogen
- Isoagglutinins

For reference the various steps in the extraction have been placed in Table I Time does not permit discussion of details Suffice it to say that Step 1 yields an extract which corresponds to Cohn's fractions IV V and VI and contains albumin and alphalipoprotein This fraction will be referred to as *Fraction A* throughout and is chiefly notable in this discussion because it contains the alphalipoproteins Step 2 which yields an extract corresponding to Cohn's fraction *II* and is known here as *Fraction B contains little* except gamma globulins Step 3 results in an extract called *Fraction C* which corresponds to Cohn's III$_0$ and is chiefly notable here

because it contains the betalipoproteins Step 4 yielding *Fraction D* and containing fibrinogen prothrombin and other substances need not concern us in this discussion which will be *focussed* almost entirely upon the alphalipoproteins the betalipoproteins and their relationship to each other

TABLE II

Distribution of Protein and Cholesterol In Young Normal Subjects

		Normal Women 28 Observations 15 Subjects	Normal Men 23 Observations 20 Subjects
Total Protein (gm percent)	Average	73 65 81	74 6.5 86
Protein in A (percent)	Average Range	69 3 63 4 79 2	70.2 63 7 75 8
Protein in B	Average Range	12.1 73 152	11 5 80 165
Protein in C	Average Range	11 7 78 154	11 8 98 14.5
Protein in D	Average Range	70 3.5 12.1	C 5 49 109
Total Cholesterol (mg percent)	Average Range	200 132 258	200 136 272
Cholesterol in A	Average Range	34 4 21 1 47.5	28 2 13 3 39 4
Cholesterol in B	Average Range	49 00 84	33 09 64
Cholesterol in C	Average Range	59 1 43 8 67 1	68 6 55 7 84 5
Cholesterol in D	Average Range	30 02 11 7	27 10 72

The results on a series of young normal men and women are shown in Table II It will be seen that value for total protein and the protein in the A B C and D fractions average about the same for normal men and normal women Values for total cholesterol are approximately the same for the two sexes Differences however are apparent in the distribution of cholesterol the average

and range of values in Fraction A being higher in young women with correspondingly lower values in the combined Fractions B, C, and D

For purposes of this discussion we are assuming that all of the cholesterol in Fraction A is in the form of alphalipoproteins and all of the remainder is in the form of betalipoproteins

Table III shows in simplified form differences between normal individuals and cases of atheroma, diabetes, and nephrosis Several differences will be noted The protein in Fraction A is lower both in atheroma and in diabetes, and very much lower in nephrosis The protein in Fraction C is higher in atheroma and diabetes and is enormously increased in nephrosis

The total cholesterol averages higher in atheroma and in diabetes than in normal individuals but is subject to large variations in all groups In nephrosis values for cholesterol are enormously increased

More striking changes are apparent in the distribution of choles terol In the atheromatous patients a reduction is apparent in the average as well as in the range of values of cholesterol in Fraction A The same change is less constant but often quite as marked in the diabetics and is extreme in the nephrotics

Scrutiny of Table III might suggest that decrease in the percentage of the cholesterol in Fraction A, representing a decrease in the amount of alphalipoprotein, might be implicated in the pathogenesis of atheroma With equal validity one might regard as pathogenetically significant the corresponding increase in the cholesterol in other fractions presumably representing the increase in betalipoprotein or might think that the distribution and propor tions of alpha and betalipoproteins were determinant in solubility or precipitability of lipid substances in the blood and hence in the deposit of these substances in tissues

These thoughts are more intriguing in the light of information now available concerning the composition of alpha and betalipo proteins Oncley s analyses(2) of betalipoproteins have indicated that the compound may contain cholesterol and phospholipids in approximately equal amounts by weight indicating a 2 1 molar ratio of cholesterol to phospholipid Less is known of the character istics of alphalipoproteins but preliminary studies have indicated probable combinations of cholesterol and phospholipids in a 1 1 molar ratio In studying the distribution of these two groups of substances it seems possible that we are estimating indirectly

TABLE III

Comparison of Normal Individuals Atherosclerotics, Diabetics and Nephrotics

		Normal Women 28 Observations 15 Subjects	Normal Men 23 Observations 20 Subjects	Atherosclerosis 25 Observations 25 Subjects	Diabetes 37 Observations 33 Subjects	Nephrosis 13 Observations 11 Subjects
Total Protein (gm percent)	Average	7 3	7 4	7 2	7 0	4 2
	Range	6 5 8 1	6 5 8 6	6 1 8 1	6 2 7 9	3 2 5 0
Protein A (percent)	Average	69 3	70 2	61 4	62 5	34 8
	Range	63 4 79 2	63 7 75 8	55 4 69 6	37 7 73 1	13 7 48 4
Protein in C (percent)	Average	11 7	11 8	14 8	17 3	43 7
	Range	7 8 15 4	9 8 14 5	10 1 24 1	9 3 39 7	33 1 65 9
Total Cholesterol (mg percent)	Average	200	200	253	247	577
	Range	132 258	136 272	135 452	95 794	354 1183
Cholesterol in A (percent)	Average	34 4	26 2	13 9	20 6	5 5
	Range	21 1 47 5	13 3 39 4	5 6 25 6	5 6 39 7	1 8 9 6
Cholesterol in BCD (percent)	Average	65 6	73 9	98 1	79 4	94.5
	Range	78 8 52 5	86 7 60 6	94 4 74 4	94 4 60 3	98 2 90 4

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TABLE IV

Cholesterol Phospholipid Ratios in Plasma and Fractions A and C

Subject	Sex	Age	Diagnosis	Total Cholesterol mg	Total Phospholipid mg	CHOL/PL RATIO Total Plasma	Fraction A	Fraction C
Bu	F	34	Normal	142	151	0.94	0.69	1.39
We	F	25	Normal	170	162	1.04	0.56	1.45
Wi	F	25	Normal	156	167	0.95	0.57	1.30
Le	F	25	Normal	178	200	0.89	0.46	1.19
Pa	M	24	Normal	176	173	1.00	0.52	1.41
Ge	M	44	Xanthoma	398	292	1.36	0.46	1.46
Ha	M	39	Coronary	218	210	1.08	0.31	1.35
Gu	F	24	Diabetes	781	519	1.43	0.64	1.61
Ma	F	21	Diabetes	314	267	1.18	0.19	1.53
Po	M	55	Diabetes	260	313	0.83	0.57	1.29
We	F	64	Diabetes	231	251	0.91	0.42	1.27
Cl	M	23	Nephrosis	394	325	1.21	0.50	1.44
Co	M	28	Nephrosis	632	475	1.33	0.43	1.51
Sm	M	55	Nephrosis	648	450	1.44	0.45	1.51
Ro	F	60	Myxedema	374	360	1.02	0.51	1.27
Sm	F	45	Myxedema	595	481	1.24	0.52	1.50
Pe	F	68	Biliary obst	675	1375	0.49	0.42	0.53
Me	F	54	Biliary obst	375	658	0.57	0.42	0.63
Ku	F	50	Biliary obst	354	610	0.58	0.51	0.90

cholesterol phospholipid relationships and that when our analyses in atheroma, diabetes and nephrosis indicate a low percentage of alphalipoprotein, the cholesterol phospholipid relationship will be high, and when, as in healthy young women the percentage of alphalipoproteins is high the cholesterol phospholipid ratio will tend to be low

This would appear to be a matter of some significance since recent publications of Ahrens(3) Kellner(4) and Gertler and Garn(5) have emphasized the relationship of cholesterol to phospholipid in the solubility of lipids in serum and in the pathogenesis of atherosclerosis

The hypothesis that the distribution of cholesterol in these various fractions of the Cohn Method and the relative concentration of alpha and betalipoproteins affect cholesterol phospholipid ratios can be validated only by simultaneous analysis of both cholesterol and phospholipids in the whole serum and in each of the fractions We have carried out such examinations in normal individuals and in a variety of pathological states

In Table IV some of our analyses have been included One sees that in a wide variety of conditions the values of the cholesterol phospholipid ratio range around 0 50 in Fraction A and around 1 35 in Fraction C The only exceptions thus far encountered have been in biliary obstruction where one finds cholesterol phospholipid ratios in Fraction C not grossly higher than those in Fraction A

Gofman Are these weight ratios?

Barr Yes all of the values in the table represent weight ratios

It is significant however that the average weight ratio of 0 50 for Fraction A indicates a molar ratio of 1 1 while the average weight ratio of 1 35 for Fraction C denotes a molar ratio considerably greater than 2 1 Furthermore wide variations in the ratios of both fractions are apparent – from 0 34 to 0 69 in Fraction A and from 1 07 to 1 61 in Fraction C

The analyses indicate clearly that a high percentage of alpha lipoprotein such as is encountered in the plasma of normal young women will tend to produce a low cholesterol phospholipid ratio and that the low values of alphalipoprotein in atheroma and nephrosis will tend to raise cholesterol phospholipid ratios On the other hand the highly variable values for the ratio of each fraction

Kellner We have not done lipoprotein studies on the tissue fluids we have obtained In that regard I should like to ask Dr Barr whether this method or a similar method for fractionation of lipoproteins has ever been applied to animal fluids animal sera or animal lymph, or has it been limited thus far to human sera?

Barr The Cohn fractionation was developed empirically to meet the special conditions of the proteins of pooled human plasma Ionic strength pH the other factors in the reaction mixture must be modified if other proteins are included There was no certainty that the methods which Cohn developed for pooled normal plasma would be applicable to the separation of proteins of abnormal plasma In this connection it is of special interest to us that separation of the highly abnormal proteins of multiple myeloma may be imperfect with the reagents published in Method 10 It is indeed a matter of good fortune that the proteins which occur in the plasma of diabetics coronary patients and xanthomas may be effectively separated It would be surprising if the solutions used in the extractions of Method 10 were applicable to the separation of the plasma proteins of species other than man

Simms Dr Barr you have stressed particularly in your study of the lipoproteins the cholesterol present in these fractions Have you made any studies of lipoid materials other than the cholesterol?

Barr Only the phospholipids We are now starting analysis of other lipid substances

Simms The deposition in atherosclerosis is not limited to cholesterol but also involves the deposition of neutral fat particularly in the early plaques

Shorr Dr Barr when you carry out repeated studies on the same individual do you find appreciable variations or are the values prone to fall within a narrow range around the average? Also have you studied any clinical condition in which you have attempted to alter the state of the disease such as in diabetes?

Barr We have studied repeatedly the plasma of several normal individuals The distribution varies but within narrow limits We have repeated tests again and again on one diabetic girl whom we found originally to have a very low percentage of cholesterol in Fraction A In spite of modifications in diet and in the dosage of insulin her abnormalities have persisted with little variation

Stamler Did you attempt to assay the presence of atherosclerosis in cases of biliary obstruction with hypercholesteremia?

distort this simple relationship It is apparent that cholesterol phospholipid ratios for the whole plasma can not be predicted exactly by determinations of the distribution of cholesterol between the two types of lipoproteins

To summarize, the results here reported indicate clearly that the distribution of proteins and lipids in the plasma of athero sclerotics diabetics, and nephrotics may differ significantly from that in young normal individuals, furthermore, that the pattern of distribution in the three conditions associated with extensive lipid deposit is qualitatively quite similar

While the disturbance in the distribution of cholesterol may be regarded as the most prominent abnormality disclosed by our observations it is seldom apparent without an accompanying decrease in albumin and an increase in the total protein in Fraction C It is not permissible to consider one change as separate or unrelated to the others It is evident that whatever the significance of the abnormalities may be, they are complex and involve factors other than lipids and lipoproteins The relationship of these changes to the pathogenesis or diagnosis of atheroma is by no means estab lished by the present data The number of examinations are not sufficient More normal controls are needed The number in the series of young men and particularly young women who survived myocardial infarctions is not sufficient Furthermore it is possible that the pattern of distribution encountered in these conditions, that is coronary disease diabetes and nephrosis, may not be specific but may represent a general pattern of response of the body to a variety of diseases or insults The influence of such factors as infections malnutrition, dietary excesses, gonadal hor mones and other endocrinal factors have as yet been insufficiently evaluated

Nevertheless I think it is apparent that future efforts to relate the lipids of plasma to the deposit of lipids in tissue must take account of their combinations with protein and particularly with the two groups of compounds that we now call alphalipoproteins and betalipoproteins

Fremont-Smith Dr Barr I should like to ask whether there is any indication that this relationship of lipids to protein also exists in the tissues? Do we know whether lipid deposits in tissues are tied in with proteins as they are in the plasma?

Barr There is as yet no information on this important question Dr Kellner is approaching it with his studies of tissue fluids and lymph

Gofman In other words the absolute values won t show the difference as clearly as this percentage will?

Barr That is right, and I would repeat that there are instances in which the percentage of cholesterol in Fraction A is low even with low total cholesterol

Gofman In Fraction A there is cholesterol alpha globulin and lipoprotein With a given cholesterol level assuming you knew the components of alpha which consists of at least three different molecules you could calculate the alphalipoprotein However that could be influenced markedly by the amount of albumin still present

Barr I may state that much difficulty has been *encountered* in separating completely albumin from alphalipoprotein

Gofman The two can be effectively separated by the ultra centrifuge You would have to use heavy water plus salt because of the high density of the alphalipoprotein

Kendall It seems to me that the significance and importance of these observations of Dr Barr s is increased if one considers that blood serum is not an active tissue It is not a living tissue in the sense that the other tissues in the body are The serum is a trans port mechanism and the level of any substance in the serum is determined by what goes on elsewhere in the body the level is determined by the balance between the processes which build up and break down the substance An increase or a decrease in the amount of the alphalipoprotein the stablest form in which choles terol is carried in the serum seems to me to indicate very profound changes in metabolism *elsewhere in the body* I think we are placing too much importance upon serum levels as etiological factors I think that in this problem we should go beyond the levels and focus our attention upon the factors which determine these serum levels

Considered from that point of view the data presented by Dr Barr indicate that individuals with arteriosclerosis have metabolic derangements that are reflected in the serum proteins as well as in the serum lipids It seems to me that these results may point the way to getting arteriosclerosis out of the serum lipid field and back into a study of general metabolism

Wakerlin I should like to ask Dr Barr a question You have relatively limited data on aging in relation to your normal indi

Barr They all survived, so that we could not determine by autopsy the extent of their lesions, but they had no clinical evidence of atherosclerosis The serum of each one was crystal clear, although in one instance the cholesterol value was 775 and the phospho lipid value was nearly 1375

Wakerlin Have you made any studies of free cholesterol versus cholesterol esters, and if so, are there any important differences in the alpha and betalipoprotein complexes?

Barr No we have not, although we are about to start such analyses

Shorr Has insulin any effect on these findings in diabetics?

Barr In the diabetic girl whom we studied so extensively we varied the insulin dosage without significant change in the distribu tion of cholesterol

Fremont Smith Have you had an opportunity to study such a patient with a profound hypoglycemia as occurs during the acute phase of an insulin reaction?

Barr We have only one observation on hypoglycemia No alteration in distribution of cholesterol was noted

Dock The betaglobulin tends to drop rather than the alpha — where there is a change in the ratio Where the alpha is low the absolute drop is not very low but the beta tends to rise The alpha globulin diminishes

Barr I don t know which is the cart and which is the horse

Dock But the beta globulin diminishes absolutely in these cases with low cholesterol content

Barr Low percentages of cholesterol in Fraction A are not limited to the high values for total cholesterol The averages do not show the relationship but there are instances where the choles terol in Fraction A is absolutely below the values in normal individuals

Gofman If you plot the data how does it come out in terms of the absolute cholesterols? Would they be in general lower in A in the diabetics and the normals? The diabetics on the average do not show it, nor the atheromatics but in the nephrotics it is striking

Barr When the total cholesterol is constant the cholesterol in Fraction A will be lower in atherosclerotics diabetics and neph rotics than in normals

Wakerlin Conversely one could have a large change in the rate of input and a large change in the rate of utilization without any change in the serum content

Kellner I should like to come to Dr Kendall's side of the discussion I agree with him that to a certain extent the level of lipids in the blood or of particular lipid aggregates in the blood may be overemphasized as far as the pathogenesis of atherosclerosis is concerned In our experimental studies of lipids in tissue lymph we have found that in many instances there is no relationship between the lipids in the blood and the lipids in the tissue lymph One may find enormous amounts of a particular lipid in the blood and yet little or none of that lipid may traverse the endothelium into the tissue lymph

It should be borne in mind that atherosclerosis does not occur in the bloodstream The atheromatous plaque is morphologically outside the bloodstream and the lipids that are deposited in the atheromatous plaque are lipids that have crossed a presumably *intact* endothelial membrane Before we can say that any lipid or group of lipids is pathogenetically related to atherosclerosis it would be important to know the permeability of the endothelium to that lipid under normal and pathological conditions

Simms In my paper this afternoon I shall show that lipfanogens penetrate the endothelial membrance of arteries *in vitro*

Barr I believe Dr Kellner that in observations on your experimental animals you have shown that values for serum cholesterol and serum phospholipid may rise *without* any increase in the concentration of these substances in the lymph which you withdraw at the same time You demonstrate atherosclerosis in your animal The serum is abnormal and the lymph is normal and yet you suggest that the atherosclerosis is dependent upon composition of the lymph How does this happen?

Kellner The lymph obtained in our experiments was tissue lymph which is quite different from the thoracic duct lymph that has usually been studied in the past The lymph obtained by cannulation of subcutaneous lymphatics of the lower extremities of rabbits represents fluid that had not gone through even a single lymph node The lipids of this tissue lymph are according to Drinker a reliable measure of the lipids of the tissue fluid

Normal rabbits that were fed cholesterol were found to have a great increase in the amount of lipid in the blood serum and also

viduals, but you did say that you have some data on older individuals. As the normal human ages, do you find a shift in the cholesterol linked up with betalipoprotein as compared with alphalipoprotein?

Barr: Our data are insufficient to answer this question. We have studied men of sixty who had the pattern of young men, but we have not attempted to collect a group of so-called normal "old men" for comparison with those who, from a clinical standpoint, are more obviously arteriosclerotic. The difficulties of establishing normality appear to increase with age.

Wakerlin: That is true, but I think it is just as important to have data on so-called normal aging individuals as it is in so-called young individuals.

Fremont-Smith: Have you studied patients with Addison's disease?

Barr: Yes, we had one, who showed no abnormalities in distribution.

Gofman: I should like to raise a philosophical point in reference to Dr. Kendall's statement. I think it is entirely likely that there can be a profound alteration in tissue metabolism. On the other hand, one must accept the possibility that blood lipids in transport represent the source of lipids in the development of atherosclerosis. The exact blood level of a certain type of lipid would then be of the highest importance.

If one takes any component, A or X, in the blood, and wants to know about its level, that level, as Dr. Kendall said, is determined by all the factors which pour that substance into the blood and by all the factors or processes which take that substance out of the blood. As a result of this balance, one achieves a relatively steady state of this substance. However, with only slight alteration in the input rate or in the output rate of any one substance, the steady-state level can be changed considerably, even by a factor of 2. This might be a metabolic alteration of not too great consequence in terms of tissue lipid metabolism, but still giving rise to a two-fold alteration in blood level. I think we could go back on the track that the serum level is important because atherosclerosis may be, after all, an accidental incident, relative to the transport of blood lipids rather than something fundamental in a metabolic sense. There need be no inconsistency in this with the view that the metabolic derangement leading to high blood levels is equally important.

Wakerlin Conversely one could have a large change in the rate of input and a large change in the rate of utilization without any change in the serum content

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Normal rabbits that were fed cholesterol were found to have a great increase in the amount of lipid in the blood serum and also

in the tissue lymph It is noteworthy that the changes in the cholesterol phospholipid ratio that occurred in the serum were also present in about the same degree in the tissue fluid These animals developed atherosclerosis In rabbits with alloxan diabetes, on the other hand, there was disparity between the lipids in the blood serum and those in the tissue lymph It is well known that when rabbits are made diabetic with alloxan, about a third of the animals have a striking lipemia after three or four days the blood lipids reaching 3 000 mg per 100 cc or higher At the time that the blood lipids in these alloxan diabetic animals were at these extremely high levels the lipids in the tissue lymph were found to be essentially normal This indicates that the increased lipids in the blood serum did not cross the endothelium and did not get into the tissue lymph We have not fractionated these sera and we do not know the physical chemical state of the lipids It is of considerable interest in this connection however that Duff has demonstrated that alloxan diabetic rabbits were quite resistant to atherosclerosis, and it is possible that this altered permeability of the endothelium to lipids may play a role in the failure of these animals to develop atherosclerosis

Goldblatt Isn t the important point the question of deposition of lipids directly from the blood or indirectly from the tissue fluid? The likelihood is that in the case of the aorta at least the deposition in the intima might be directly from the blood stream rather than indirectly from lymphatics or vasa vasorum

Shorr Is that necessarily true? Do you refer to the direct contact between the blood and the surface of the arteries or to the contact between the artery and the fluid that has passed through the capillaries of that artery? In the latter instance Dr Kellner s observations would still hold for large vessel atheroma Am I wrong in this interpretation?

Goldblatt I don t think that the answer is definitely known I should like to have discussion on whether you think deposition occurs directly from the lumen of vessels or whether you think it comes by way of lymphatics or capillaries in the vessel wall

Gofman We have had statements here on whether or not blood or lymph is involved because of the question of permeability I think no one has brought up any evidence — and to my knowledge none exists — on whether atherosclerosis involves the process of permeation in any sense such as filtration through a membrane or whether it involves active participation of endothelial cells in a

function other than strict permeability through a membrane with no holes so to speak Unless we have real evidence which suggests what the nature of passage of lipids is by a membrane through a membrane or into a membrane I think we are in no position to answer that question

Fremont Smith Dr Kellner in the lipemic rabbits you spoke about is the cholesterol to a considerable extent in globules or is it perhaps in the same state in the plasma as the lipids in a clear plasma? Would that make a difference?

Kellner Yes We have observed that in animals with grossly milky blood serum the tissue lymph was usually crystal clear The large lipid aggregates the chylomicrons did not appear to traverse the capillary endothelium under the experimental conditions used Lipemia was produced by various means such as by injecting surface active agents and alloxan in each instance the tissue lymph obtained was water clear even though the blood serum obtained at the same time was milky

Wakerlin Shouldn t it be pointed out that actually what you are collecting is fluid that has passed through two membranes (a) the blood capillary membrane and (b) the membrane of lymphatic capillary You are not in a position to say, it seems to me that you are gathering fluid exactly similar to that in the tissue spaces The so-called interstitial or extracellular fluid may have certain differences from the lymph which you are gathering it is granted from small lymphatics since the lymph in the small lymphatics has passed through the lymphatic capillary membrane and the permeability of that membrane may be *different* from the blood capillary membrane

Fremont Smith Lipids may have been left behind in the tissue spaces after having left the capillaries and not gone back into the lymph

Wakerlin Theoretically this sounds like a valid objection but *I don t* believe it is If any significant amount of protein or lipoid were left behind it would be a matter of only a *few hours* when the tissue spaces would be loaded with such substances

Kendall A few years ago we made a study of lipemia produced in rabbits with *alloxan* Our results were similar to those described by Dr Kellner Following the administration *of alloxan* there is a steady increase in serum lipids *reaching* a maximum in from 5 to 10 days then if the animal survives a decrease to near normal

values in about another 10 days The serum lipid values remain down permanently unless the animal is placed on insulin and allowed to build up stores of body fat If it is given insulin, and a good diet, the animal will gain weight Then if it is taken off insulin, a secondary lipemia develops In the animals examined after the lipemic flood was over, almost total lack of subcutaneous and visceral fat was found The lipemia, it seems to me, in these animals must be produced by the mobilization of deposits of fat into the blood stream This fat has presumably passed the membranes from the tissues into the blood stream that is the fats, cholesterol, and phospholipids can get through in one direction without apparently causing very much change in the concentration of these substances in the lymphatics Looking at them from this point of view makes Dr Kellner's observations even harder to interpret than they are from his point of view

Stamler An important experiment in this field would be chronically to maintain such an alloxan induced hyperlipemia which is endogenous in origin in contrast to that produced by cholesterol feeding Would this chronic hyperlipemia involve cholesterol? Would the plasma cholesterol/phospholipid ratio be elevated? Would atherosclerosis eventually supervene as occurs in association with chronic endogenous hypercholesteremic hyperlipemia induced in chicks by prolonged estrogen administration?

Concerning the mechanism of alloxan induced hyperlipemia it may not be exclusively a metabolic mobilization of fat stores Alloxan is a potent producer of renal damage the initial acute lipemia may be at least in part a renal hyperlipemia

Concerning the mechanism whereby lipid enters the arterial intima Pollak and Bevans *et al* produced atheroma acutely by *intravenous injection of artificial cholesterol emulsions* We attempted to repeat that modifying the experimental procedure in one respect We injected hyperlipemic plasma as our cholesterol rich emulsion That is we obtained hyperlipemic plasma from cholesterol fed rabbits or chicks and gave it intravenously to rabbits and chicks *No atheroma was observed in aortas of animals* sacrificed 6 72 hours later Multiple microscopic sections revealed no evidence of intimal phagocytosis of lipid from either homologous or heterologous plasma

Dock Okey of Berkeley California (6) noted that in rabbits fed diets containing 5, 10 15 percent of fat the cholesterol content of the carcass did not increase The cholesterol content of the

liver was not increased unless excessively large amounts of fat were administered The cholesterol content of the hairless skin however goes up progressively When you double the fat the cholesterol content of the skin doubles and when you treble the fat content of the diet, the cholesterol content of the skin triples The skin like the lining of arteries contains considerable connective tissue It would be of interest to see whether the connective tissue in the skin changes in atherosclerosis with various experimental procedures The connective tissue may accumulate cholesterol in connection with the total metabolic activity that is going on in the body and not necessarily in relation to a blood level

Stamler In rats fed a diet containing 10 to 20 percent cotton seed oil and 5 percent cholesterol the aorta cholesterol does not change over months There may be other changes but the aorta cholesterol remains at normal levels The 10 to 20 percent oil +5 percent cholesterol were mixed with a commercial chow (Purina) containing approximately 3 to 5 percent total fat

Kellner Did your animals have elevated blood cholesterol levels on that diet?

Stamler No

Kendall It is interesting in regard to this that there have been very few experiments in which the balance between cholesterol intake and cholesterol excretion has been measured in the rat Cook has obtained certain data upon it It seems that the rat differs from many other experimental animals in that a large part of the dietary cholesterol is excreted as cholesterol in the feces I think this observation explains the failure to produce striking results experimentally in the rat in this field

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BLOOD LIPIDS IN EXPERIMENTAL ARTERIOSCLEROSIS

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In the field of experimental arteriosclerosis just as in clinical arteriosclerosis we have been preoccupied with finding correlations between changes in the blood lipid pattern and the development of arteriosclerosis This preoccupation would be justified perhaps if cholesterol is considered to be an inert substance whose only metabolic fate is excretion in the feces as cholesterol or coprosterol But this preoccupation is perhaps not so logical if cholesterol is active metabolically that is if the tissues of the body possess the ability to break down cholesterol If so the study of the form in which it occurs in serum becomes important as a symptom of changes taking place elsewhere rather than being important as a primary etiological factor in the production of the disease

This morning instead of discussing our accumulated data on variations in the serum lipid pattern in rabbits and in dogs in which experimental arteriosclerosis is being studied I should like to discuss this animal work and see if it lends any support to the idea that cholesterol may be a metabolically active substance in the body whether there is any evidence that catabolism of cholesterol may be a factor that we shall have to consider

We are in a much better position today to discuss metabolism of cholesterol than we were a few years ago The fact is we are in a much better position to discuss it today than we would have been six months ago Up until newer techniques utilizing isotopes became available cholesterol was considered to be an inert substance Cholesterol is a comparatively unreactive substance outside the body Analytical methods for its determination are laborious and are not specific in their application Available data seemed to indicate that there was a rough balance between the amount of cholesterol taken into the body and the amount secreted in the feces With the development of isotopic techniques it was soon shown that the body could synthesize cholesterol that it could

build up the cholesterol molecule from simple molecules It was shown that any ingredient of the diet which could furnish two carbon atom chains could be utilized by the body for the synthesis of cholesterol and that this synthesis takes place in the liver in the spleen, in the walls of the small intestine in skin In fact, every tissue that has been adequately studied has been shown to synthe size cholesterol to a more or less active degree

The other side of the picture has not been studied at all adequately That is, practically no information at all is available as to whether or not tissue cells have the ability to break down cholesterol Part of this lack of knowledge is due to technical difficulties in the study of catabolic processes involving cholesterol Cholesterol is difficultly soluble in aqueous solvents There is no easy way of getting cholesterol into cells in isolated tissue sections so that the fate of marked cholesterol can be studied experi mentally But in the last few years considerable data have been obtained in intact animals indicating that cholesterol does have an active catabolic metabolism as well as an anabolic metabolism Rittenberg and Block showed that the administration of cholesterol marked with heavy hydrogen resulted in the secretion of bile acids containing the label They also showed that the administration of this labeled cholesterol resulted in the secretion of dihydropreg nandiol a substance derived from one of the female sex hormones The body evidently has the ability to utilize cholesterol for the synthesis of bile acids and the steroid hormones

Recently work has appeared which indicates that slices of testes *can utilize cholesterol for the synthesis of testosterone But quanti* tatively these conversions may not be important that is the body may not be utilizing large amounts of cholesterol metabolizing it through these routes However, recently Gould has reported that *intact animals can break down cholesterol completely* to carbon dioxide and water Cholesterol labeled biologically with C_{14} was fed or injected intraperitoneally into these animals and as much as 50 percent of the activity could be recovered in the expired carbon dioxide He showed in addition, that the administration of labeled cholesterol led to the excretion of organic acids con taining the label organic acids that are presumably other than the bile acids previously studied thus confirming the observation of Cook that the administration of cholesterol to rats leads to a marked increase in the excretion of an optically active C_{27} acid which presumably arises by the opening up of one of the choles terol rings

This work of Gould's does not show that this oxidation of cholesterol actually takes place in cells. It may possibly arise through degradation of the cholesterol in the alimentary canal by bacterial action. The appearance of the label in the expired carbon dioxide may be the result of the degradation of the cholesterol leading to the formation of bile acids or steroids. There are a great many unsolved problems in this field. But I think these observations, if they are confirmed, are going to force us to shift our attention in the field of arteriosclerosis from what is present in the serum to what is happening in the cells.

What information along these lines has been obtained by our animal experimentation? I shall confine my report largely to the result in the dogs rather than in rabbits, although observations made in the rabbits tend to confirm the conclusions that we are almost forced to draw from the dog. Now, you know, a dog is pretty human in so far as his serum cholesterol picture is concerned. The serum level of cholesterol in the normal dog will vary from around 120 to 300 mg percent, with an average very close to 200 mg percent, thus duplicating the picture in human beings. Of this cholesterol, about two-thirds is present *in ester* form and one third as free cholesterol. Little work has been done in studying the distribution of this cholesterol in different fractions, as has been done for human serum. We do not know whether he actually has alpha- and beta-lipoproteins, and so forth. If he does, the quantitative relationship between the cholesterol and the phospholipid in these fractions will probably be found to be different, because the dog differs in his serum from a human being. A normal human being with a cholesterol level of 200 mg percent will have 9 or 10 mg of lipid phosphorus associated with that cholesterol; a dog will have from 15 to 18 mg of lipid phosphorus. A dog normally has twice as much phospholipid as a human being. If a dog is placed upon a cholesterol-containing diet, we find that the addition of the cholesterol has comparatively little effect upon the serum level. In this respect a dog resembles a human being. If you add 100 gm of egg yolk powder to the diet of a human being, in this way adding approximately 3 gm of cholesterol a day, only a slight change in the level of total serum cholesterol is produced. If the same amount of cholesterol is added to the diet of a dog, cholesterol is raised by about the same amount. If the amount of cholesterol in the dog's diet is increased to 10 gm of cholesterol a day — and this can be done by dissolving the cholesterol in ether, pouring the ether solution over the dry diet

and evaporating off the ether, leaving the cholesterol in finally divided form — the level rises from about 200 mg percent to about 400 mg percent By keeping dogs upon this diet for long periods of time, the cholesterol level gradually drops so that at the end of a year or 18 months, the level may be only slightly increased above normal Balance experiments upon these dogs show that they are absorbing this cholesterol Dogs being fed 10 gm of cholesterol in its finally divided form will excrete between 1 and 3 gm of additional nonsaponifiable lipids I am specifying nonsaponifiable lipids here because there is a possibility that part of the cholesterol fed an animal may be converted by bacterial action in the gut to substances which have lost properties which enable them to be precipitated by digitonin or to give the color reactions used in the determination of cholesterol By speaking of increase in nonsaponifiable matter we speak of a maximum value Dogs receiving 10 gm of cholesterol will excrete only from 1 to 3 gm more cholesterol than these same dogs did upon a cholesterol free diet A dog upon a cholesterol free diet will excrete between 0 3 and 0 5 gm of sterols a day These dogs are handling from 6 to 8 gm of cholesterol with only a small increase in serum level, and in no case develop arteriosclerosis

If we administer thiouracil to a dog and depress his thyroid function the serum level of cholesterol is also increased The administration of doses of thiouracil large enough to produce a 15 to 20 percent reduction in the basal metabolism of the animal will increase the level of the serum cholesterol from 200 mg per cent to approximately 400 mg percent and dogs can be maintained in this state for long periods of time We have had them on thiouracil for as long as two years and the serum level remains pretty constant at this point without ever producing any arterio sclerotic lesions The administration of thiouracil does not influence the amount of steroids being excreted in the feces of these animals The change in level in these animals is not due to a change in the amount of cholesterol being eliminated

If to the diet of an animal on thiouracil we add 10 gm of choles terol a day the serum level rises in some cases to astronomical heights In some animals this regimen leads to serum cholesterol levels as high as 5000 mg percent The average animal in our group which now outnumbers a hundred will show a serum cholesterol level during thiouracil cholesterol feeding of 1000 mg percent Under these conditions the dogs rapidly develop arterio sclerotic lesions but these dogs are not different from dogs being

fed cholesterol without thiouracil in their ability to excrete cholesterol The excretion under the thiouracil cholesterol regimen is just the same as it was before the thiouracil was started If the dogs are kept upon the regimen for a period of from four to six months they develop a pretty consistent degree of arteriosclerotic involvement the degree of involvement being dependent upon the level of serum cholesterol maintained and the length of time on experiment If the dogs are maintained upon the regimen we will say for four months and then taken off the regimen if either the cholesterol or thiouracil is removed from the diet the serum lipids return to normal within a week If these animals are maintained upon a stock diet with normal serum cholesterol levels for a period of four months there is a rapid regression of lesions Evidently the dog possesses the ability to clear deposits of cholesterol and lipid from his arterial walls

The words of that term has the ability to clear cholesterol deposits from the arterial walls may be significant because *it is a reflection of the* earlier point of view that cholesterol is an inert substance that is not being metabolized but is being cleared There is a tendency in our minds I believe to think of this cholesterol as being mobilized from the cells back into the circulation and perhaps disposed of *there* but *is* that the actual situation? Is cholesterol is lipid being moved out of the cells or is it being used up there?

Simms May I interrupt and raise the same question that I did with Dr Barr as to whether you are referring to cholesterol only or to lipids in general or to neutral fat?

Kendall I am referring to the entire lipid in the cells

Simms Then you are not limiting this to cholesterol?

Kendall I am not limiting this lipid in the cells to cholesterol Analysis of the early lesions does confirm what Dr Simms mentioned earlier that early lesions contain large amounts of neutral fat as well as cholesterol and cholesterol esters But as the lesions regress there is a *decrease* in both neutral fat and in cholesterol and cholesterol esters

The results obtained in rabbits do not agree very well with the observations made in dogs In the first place if a rabbit is maintained upon a cholesterol containing diet for sixty or eighty days there is a general development of lipid deposits in the walls of the arteries When the animal is taken off cholesterol the serum

and evaporating off the ether leaving the cholesterol in finally divided form — the level rises from about 200 mg percent to about 400 mg percent By keeping dogs upon this diet for long periods of time the cholesterol level gradually drops, so that at the end of a year or 18 months, the level may be only slightly increased above normal Balance experiments upon these dogs show that they are absorbing this cholesterol Dogs being fed 10 gm of cholesterol in its finally divided form will excrete between 1 and 3 gm of additional nonsaponifiable lipids I am specifying nonsaponifiable lipids here because there is a possibility that part of the cholesterol fed an animal may be converted by bacterial action in the gut to substances which have lost properties which enable them to be precipitated by digitonin or to give the color reactions used in the determination of cholesterol By speaking of increase in nonsaponifiable matter we speak of a maximum value Dogs receiving 10 gm of cholesterol will excrete only from 1 to 3 gm more cholesterol than these same dogs did upon a cholesterol free diet A dog upon a cholesterol free diet will excrete between 0 3 and 0 5 gm of sterols a day These dogs are handling from 6 to 8 gm of cholesterol with only a small increase in serum level and in no case develop arteriosclerosis

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Kendall Not at all

Stamler Essentially the same applies to chickens

Wilens In cholesterol fed rabbits cholesterol deposits are found not only in the arteries but are widely distributed in such tissues as the lung liver and gall bladder Does this occur in the dog as well?

Kendall That happens in the dog too Some of our dogs maintained upon the thiouracil cholesterol regimen for two years developed xanthoma in the skin as well as lesions in the vessel walls They also deposit cholesterol in the liver spleen lungs and many other organs This cholesterol disappears in the regression experiment

Gofman The rabbit is unquestionably capable of destroying large amounts of cholesterol We have checked this by the use of tritium labelled cholesterol In balance experiments one finds that three-quarters of the cholesterol is destroyed by the rabbit not excreted There is no doubt about the rabbit's ability to metabolize cholesterol to compounds other than cholesterol and to compounds other than those containing the ring system

Katz Isn't it true then that we should not talk about qualitative differences but rather quantitative differences in rates of turnover and exchange? Of course qualitative differences in metabolism may be the basis for these quantitative differences

Gofman I should like to comment on the question Dr Wilens raised The rabbit synthesizes and destroys cholesterol just as do other animals

Dock But its blood level stays up That is the striking thing — the persistence of much higher blood levels for longer periods in the rabbit than in the dog

Ogden Do you find that the destruction of cholesterol by the rabbit is mainly in the gut or more generally distributed?

Gofman If you measure the turnover of cholesterol in any organ of the rabbit except for the brain you find that the turnover is reasonably rapid for most tissues the turnover time is a week or thereabouts This cannot be accounted for by any secretion in the gut You do not find it in the stools

Goldblatt Dr Kendall did I understand you to say that you deny the destruction of cholesterol in the rabbit?

levels stay up In one experiment that we have just finished, the average serum level maintained in a group of rabbits by feeding them 250 mg of cholesterol a day was 1000 mg percent They were taken off cholesterol and placed upon a cholesterol free stock diet In many of the animals the serum levels two months after they were taken off cholesterol were still higher than they were at any time while upon the cholesterol containing diet The average for four months off diet was 700 mg percent It is not surprising to find that the severity of the lesions in these animals was much greater after four months off the diet than it was in control animals examined immediately after the cholesterol feeding period The rabbit seems to differ from the dog in its ability to dispose of cholesterol by any route other than excretion

Katz I have two points to make – (1) The chicken is more like the dog in that hypercholesteremia disappears (plasma cholesterol levels return to normal) within a few days after cessation of cholesterol feeding in cockerels fed cholesterol for 10 weeks Definite regression of cholesterol induced lesions is detectable within 15 weeks (2) In agreement with Dr Kendall s observations the early work of Anitschkow in the rabbit also showed that regression of lesions proceeds at a very slow pace in this species Lesions do regress and even disappear in the rabbit but it takes a long time (2 3 years)

Kendall The point of the experiment that we have just completed was to test whether or not lipotropic substances such as choline and inositol had any effect upon the rate of regression of lesions in the rabbit The results of the experiment could in no way be interpreted as indicating that choline or inositol had any influence whatsoever upon the regression of the lesions I think that these results indicate that the rabbit is an unsuitable animal for the study of factors which may influence the regression of lesions

Goldblatt Does that statement apply to the dog – about the regression of the lesions as a result of the administration of choline or inositol?

Kendall That also applies to the dog We have been able to demonstrate no effect of choline or inositol either on the rate at which dogs form lesions or on the rate at which lesions regress

Kellner Dr Kendall did the administration of choline or inositol to either rabbits or dogs affect the blood phospholipid levels?

getting thiouracil you would expect no change in basal metabolism If the cholesterol metabolism is affected by changes in basal metabolism then you would expect to find no change

Dock The change in basal is only 15 percent which does not strike one as very great especially in an animal such as the dog in which the environmental temperature affects the metabolism Such a change would seem to be essential when the drop in cholesterol occurs in a week I do not believe the thyroid recovers in a week after a long course of thiouracil feeding It therefore seems essential to know whether thiouracil has a specific effect on levels of blood cholesterol

Kendall I think you must be right

Stamler We attempted to raise the BMR with dinitrophenol The effect on hypercholesteremia in cholesterol fed chicks is entirely different from that of thyroid Desiccated thyroid markedly depressed hypercholesteremia and retarded atherogenesis Dinitrophenol failed to exert either effect

Kendall If you treat a rabbit with large amounts of thyroid you can prevent both the hypercholesteremia and the development of further arteriosclerosis No balance experiments have been done in the rabbit

Gofman In connection with the question raised by Dr Dock in his discussion as to whether it is thyroid or not isn t it also true that thyroxin has the same effect that thyroid has although there was some doubt as to whether the total effect was due to the thyroxin content of the thyroid extract? But certainly the major effect is due to thyroxin isn t that correct?

Kendall That is right

Gofman This was on prevention of hypercholesteremia in the rabbit

Stamler Some evidence is available that thyrotropic hormone does not prevent this hypercholesteremia

Kendall I think it is difficult to explain the observations in the dog without assuming that cholesterol is a substance which has an active metabolism in the body that is a substance that can be broken down to substances that no longer possess the properties of sterols It is true that this point of view does take the emphasis off the serum lipids to some extent but it is true that the lesions

Kendall No I consider the rabbit to be an unsuitable animal at least for this type of experiment, where we are depending upon gross observations to get evidence for the destruction, the metab olism, and the mobilization of cholesterol from the arterial lesions

Katz When hyperlipemic plasma (cholesterol levels of 1000 mg percent or greater) is obtained from cholesterol fed chicks or rabbits and given intravenously in proportional doses on a body weight basis to rats rabbits, and chickens, species differences are demonstrable in the time required for the plasma cholesterol levels to return to control values — 12 hours in rats 24 hours in chickens, and 72 hours in rabbits

Shorr Is it feasible to raise the serum cholesterol by thiouracil to a greater degree than you have customarily done? Is it possible with this adjuvant to attain the values of about 1000 mg percent which you can obtain by the feeding of cholesterol?

Kendall We have never been able to do it We have increased the thiouracil to the limits of the animals tolerance for it without being able either to depress the basal metabolism below 15 to 20 percent or to increase the cholesterol levels

Shorr I have in mind the possibility that cholesterol arising from endogenous metabolism might be transported in the blood stream in some quite different fashion from exogenous cholesterol

Kendall One point that Gould made in his report in Chicago in November might be suggestive along that line He administered radioactive acetate to rats He sacrificed the animals three hours after the administration of the acid and analyzed the various tissues for labeled cholesterol The highest concentration of labeled cholesterol that is of newly synthesized cholesterol was found in the bile The percentage of labeled cholesterol in the bile was higher than in the liver in the bloodstream or in any other tissue It seems to me this might be interpreted as indicating that the newly synthesized cholesterol passes from the liver into the alimentary canal through the bile before it becomes part of the systemic cholesterol If that is true the entire difference between endogenous and exogenous cholesterol disappears

Dock If you feed the dogs thyroid with the thiouracil nothing happens?

Kendall We have not actually done the experiment but judging from reports on the effect of thyroid administration to animals

substances or groups of substances and that one or two of these groups of substances may be responsible directly for the fat deposition but that the other ten or eleven might not be directly responsible?

Kendall I should think that could be freely conceded

Simms I think then that we are in agreement

Gofman One of our group Max Biggs has fed labelled cholesterol using tritium labels and we can say definitively that 80 to 90 percent at least of the cholesterol in atheromatous lesions in the rabbit comes from exogenous cholesterol We cannot prove that it came to the lesion via the blood We do know that the exogenous cholesterol gets into every component of the blood that we have studied and that the cholesterol in the lesion is primarily exogenous by direct demonstration of the radioactive label It still does not prove that the blood was the source although we suspect this to be the case

Stamler Couldn t the administered labelled cholesterol have been broken down and resynthesized?

Gofman I think that is highly unlikely because if the cholesterol had been broken down and resynthesized it would have been diluted so much that we would never have detected it in the lesion

Dock Dr Kendall I think it would be well to clarify the statement about fast moving molecules Do you mean fast moving in a centrifuge and not fast moving in the physicist s sense of rapidly diffusable?

Kendall No they rise rapidly in the ultracentrifuge and perhaps you might go a step farther and say that these are fast moving molecules in the plasma that is if they are transport molecules

Dock They rise in the ultracentrifuge They are therefore light molecules of considerable size

Gofman The molecules that Dr Kendall refers to as fast moving have the same relationship to the normally occurring molecules in the dog as they do in the rabbit and the human in that they have lower density than the normal lipoproteins Dr Kendall sent us some sera and by studying the rate of flotation versus medium density one can determine lipoprotein density In Dr Kendall s animals which were normal dogs or dogs on thiouracil alone or cholesterol and thiouracil they are predominantly of lower S_f classes than the S_f 10 20 group

cannot be produced in the dog or have not been produced in the dog, without a corresponding increase in serum cholesterol levels

Dr Gofman has been kind enough to run ultracentrifuge studies upon sera from our dogs The normal dog does possess molecules which move at rates corresponding to the betalipoprotein of human beings When animals are placed upon cholesterol or upon thiouracil the amount of this fraction increases enormously, but there is very little evidence that in these animals there is any increase in the amount of the S_f 10 20 fractions or of the higher faster moving molecules But when the thiouracil and cholesterol feeding are combined, then there is a shift in pattern most of the cholesterol in the serum is there in the faster moving particles The substances that Dr Gofman is studying contain neutral fat in addition to cholesterol and phospholipid It seems reasonable to regard these substances as part of the mechanism for the transport of fat If you reduce the ability of the tissue cells to utilize fat, that is, to metabolize neutral fat as well as cholesterol then you would expect that these transport substances would pile up in the serum So this shift in serum lipid picture is just as reasonably explained from the standpoint of tissue metabolism as it is from any other hypothesis This hypothesis that changes in the tissues are responsible for the development of arteriosclerosis certainly fits the observed facts in clinical arteriosclerosis better than does the assumption that the lesions in arteriosclerosis are due to the presence of abnormally high or abnormal components in the serum

Simms May I comment on this discussion and present a different point of view? As I understand it the changes observed in the plasma constituents in hypercholesteremia may merely be a reflection of a metabolic upset or a failure to maintain a balance in regard to these substances and that they in themselves may not necessarily be the direct cause of the fat deposition Is that right?

Kendall That is right

Simms I think you will admit though that the actual fatty material which is deposited presumably comes from the blood stream

Kendall I will admit that it is logical to believe that that is the source of the material in the cells but no one has ever shown conclusively that it is true

Simms Will you concede then that in conditions of hypercholesteremia there may be an upset in the balance of say a dozen

substances or groups of substances and that one or two of these groups of substances may be responsible directly for the fat deposition but that the other ten or eleven might not be directly responsible?

Kendall I should think that could be freely conceded

Simms I think then that we are in agreement

Gofman One of our group Max Biggs has fed labelled cholesterol using tritium labels and we can say definitively that 80 to 90 percent at least of the cholesterol in atheromatous lesions in the rabbit comes from exogenous cholesterol We cannot prove that it came to the lesion via the blood We do know that the exogenous cholesterol gets into every component of the blood that we have studied and that the cholesterol in the lesion *is* primarily exogenous by direct demonstration of the radioactive label It still does not prove that the blood was the source although we suspect this to be the case

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Kendall I have not had a final report from Dr Gofman on this point Apparently the chief difference between the fast moving molecules in this group, that is, the S_f 50 70 molecules and the slower ones, the S_f 10 20 ones, is in the content of neutral fat The ratio between cholesterol and phospholipid is about the same in the fast ones as it is in the slow ones?

Gofman Yes

Kendall In some fractionation experiments that we did on similar plasma here, we found the same thing Although we could get off fractions that contained as high as 85 percent neutral fat and fractions that contained as low as 30 percent neutral fat, the ratio between cholesterol and phospholipid in these two fractions was about the same That suggests to me that the relative stability of the serum content of the S_f 10 20 fraction may be related to the fact that it has a low neutral fat content as compared with the others, and it would be the fraction that would be least affected if the system was called upon to transport large amounts of neutral fat arising from the diet After a fatty meal when much neutral fat is being transported in the plasma you would expect to find a greater increase in the molecules containing large amounts of neutral fat than you would in those containing a small amount

Gofman This has also been observed

Kendall This talk has sort of merged imperceptibly into the discussion and I dont know whether I should try to say anything to summarize my ideas on the subject or not But I think perhaps I should apologize for taking advantage of this meeting as a sound ing board for many speculative ideas Although they seem to me to be important factors to be considered in planning further work I will freely admit that many of my statements rest upon logic rather than observation, and I will hold no brief for the validity of my reasoning processes

Goldblatt It may be a comfort to you to know, Dr Kendall that in the mode of your presentation you have conformed to the exact requirements of this conference

Perera Is it possible returning to the various molecules in alpha and other lipoproteins that cholesterol remains inert and that the variable is the molecular structure and characteristics of the pro tein with which it is bound?

Kendall In other words lipids bound to proteins can be metabolized whereas the lipids in these other molecules are not broken down?

Perera Or that the rate of synthesis or breakdown might depend to some extent on the character of the protein with which it is bound

Kendall I dont know Dr Gofman has the only available information about the relative rates of turnover in these different fractions

Gofman We know that all these molecules have a transitory existence in the system and I think the greatest mistake is to regard any lipoprotein molecule that bears cholesterol as having anything but a transitory existence What we measure is a serum level which is simply the balance between what is going in and going out We can say that all the molecules transporting choles terol in the system have rates of disappearance of the order of minutes hours and days There are different components dis appearing at different rates but those different rates are not of high orders of magnitude Is would appear that all of these mole cules transport cholesterol in association with protein and lipids and have turnover times in the order of hours although there are some that may be longer However a difference of one hour versus eighty minutes can mean all the difference in the world in terms of blood level.

Wakerlin As I recall Dr Kendall you and your group when first reporting found that it took some twelve to fifteen months of thiouracil cholesterol feeding in dogs to obtain gross lesions Now you obtain them in as little as two to four months What are the differences responsible for the shortening of this time interval?

Kendall When we started out on this work we had very limited facilities for maintaining dogs for doing experiments and in the first experiments we ran the dogs for fourteen or sixteen months in order to push the experiment It was only after we had demon strated that we could actually produce lesions in the dogs by this means that we began to study the length of time required to produce early lesions We could not afford to start out with a limited number of dogs and sacrifice some at the end of two months or at four months in order to see whether or not lesions were developing because that would mean that we would have to start over again and we never would have completed an experiment We started out with the long experiment first and went to the short experiment afterwards

Wakerlin As I shall report tomorrow our research group has repeated your work and has been able to confirm it but we find

with a somewhat similar technique that we get few gross lesions in four to six months in normotensive dogs I wonder what the difference might be due to We have had cholesterol levels in the neighborhood of those that you report We have not seen 5000 but we have seen 3200 mg percent and the average is about 1000 mg percent

Kendall It has been our experience that if you can maintain the level at 1000 mg percent or above you get a reproducible total involvement It has become routine in our laboratory for the pathologist to evaluate the extent of gross lesions in eight different sites and to grade them from zero to four plus In some of these animals we may find a four plus lesion in one area and practically no lesions elsewhere

Goldblatt Do you mean eight different sites in the aorta, or do you mean eight different sites in the body?

Kendall In the body The sites as I recall them are the coronary arteries the sinus of Valsalva the cerebral arteries the carotid the thyroid the lower abdominal the iliac This difference in distribution seems to me to support the idea that the defect is in the cell rather than in the serum All the arterial walls are being exposed to the same serum

Simms Isn t it possible that the factors causing the fat deposition are in the serum but that certain tissue areas are more susceptible?

Kendall That seems to me to come back to the same idea that if certain cells are more susceptible then the defect lies in those cells The difference between those cells and the cells that are not affected is certainly a factor to be considered

Simms Yes it is a factor but not the whole cause if the fatty material comes from the serum The difference between the cells would be present in a normal individual as well as in an arteriosclerotic individual It would appear to be merely a predisposing influence

Gofman There is nothing mutually exclusive about these ideas If one has an abnormality in the blood it does not necessarily indicate that arteriosclerosis will develop Nor does it exclude the possibility that the blood is the source when arteriosclerosis does occur No one has seriously doubted the local factor in arteriosclerosis It is well established that arteriosclerosis is a focal process

Kendall If the S_f 10 20 fraction and the other fat containing particles of serum are part of the normal transport mechanism of the body a normal individual who has a very low concentration of S_f 10 20 may actually be moving more cholesterol through the blood stream in that fraction than is a person whose level is high The first individual may be utilizing these particles as fast as they are being formed The other individual cannot utilize them at a normal rate therefore the level increases This view tends to take the onus off the S_f 10 20 molecule The S_f 10 20 molecule is not necessarily abnormal It is probably a perfectly normal molecule that we are continuously utilizing

Wilens If atherosclerosis is considered to result from a primary metabolic disturbance of cholesterol as you suggest it would be most remarkable to have this material deposited almost exclusively in arteries There are a number of so called lipid dystrophies some of which involve disturbances in cholesterol metabolism such as Hand s Schueller s Christian s disease in which arterial deposits do not occur In such conditions the reticulo-endothelial system is often the chief site of deposition

Kendall Then you would interpret those diseases as being metabolic disturbances and localized in certain tissues

Wilens I am not quite certain whether these diseases are clearly metabolic in origin although they are commonly regarded as diseases of disturbed metabolism In any event in these conditions abnormal lipid substances are deposited in various sites but show no predilection for arteries

Katz I want definitely to take exception to the concept that atherosclerosis is an inevitable aging process The available facts amply refute this stubborn dogma These facts support the philosophy so essential for us as investigators that this is a disease we can do something about An important concept recently appreciated in atherosclerosis research is that we are dealing with a metabolic disease in all probability With that goes hope for ultimate prevention and cure

Lansing I must insist that we are scientists first and philosophers second that we are seeking to establish the facts of the case whether we like them or not If this pattern is one of inevitability we must accept it I have never expounded the idea that arteriosclerosis is an inevitable sequence of life It usually accompanies the aging process Whether we can modify that or not is another matter

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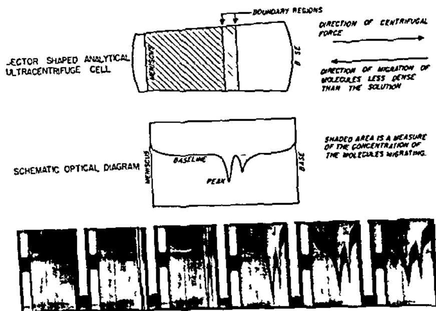


FIGURE 1 Schematic diagram of ultracentrifuge cell showing migration of molecules less dense than the solution against the centrifugal force The schematic optical diagram demonstrates the type of optical pattern obtained Below the schematic optical diagrams are actual photographs taken during an analytical run There are two major species floating with different migration rates the larger S_f 12 4 and the smaller S_f 6 2 (rotor speed 52 640 rpm) Reprinted from article by Gofman *et al* *Circulation* 2 *164* (1950)

sucrose or glucose addition and floating the lipoproteins or sinking the heavier proteins One can thus concentrate the lipoproteins and study them in an isolated fashion The actual analytical scheme is shown diagrammatically in Figure 1 When lipoproteins are present they will start out at the base of the cell and migrate against the centrifugal force The optical pattern shows a base line plus inverted peaks each peak represents a species of definite migration rate Actual photographs taken at various times at full rotor speed give evidence of the migration of the molecule

Figure 2 shows isolated fairly pure lipoprotein species from human blood The upper pattern shows a molecule that moves against the centrifugal field with a rate of 13 S_f units (Svedbergs of flotation) The middle pattern shows a molecule moving with the S_f rate of 6 units The lowest pattern shows a mixture of S_f 6 and S_f 13 molecules The S_f value is simply a measure of how fast the particular molecular species migrates If the conditions are kept standard by using the same solution composition the same

LIPOPROTEINS AND ATHEROSCLEROSIS*

JOHN W GOFMAN

Division of Medical Physics, Donner Laboratory
University of California

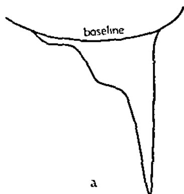
WITHOUT GOING into all the evidence that has been gathered both by our own group and others I should like to state one fact — at least as far as facts can be stated — and that is the cholesterol in the blood cholesterol esters, and neutral fats do not circulate as the molecules themselves By ultracentrifugation one can through raising the density of serum float all the lipoproteins in a serum sample to the surface of an ultracentrifugal tube If one takes these lipoproteins off the top, less than 5 percent of the lipids are left in the tube In other words essentially all the lipids in the blood are in the form of molecules of molecular weight of 80 000 or higher, and all of them are in the form of lipoprotein molecules of relatively low density densities running 1 14 or less

We have been interested for the last few years in the possibility that one might see something of consequence with respect to atherosclerosis if one could characterize the lipoproteins in a person s blood adequately that might be missed if one studied just one of the building blocks say fats or cholesterol cholesterol esters or phospholipids The ultracentrifuge is probably at the present time the instrument most capable of allowing us to examine the lipids and lipoproteins in their native state without any appreciable chemical alteration *The lipoproteins in the serum* both in the human and the experimental animal show a rather complex picture but some of the features follow well defined patterns and one can make some general statements about them

I believe almost everyone here is familiar with the general ultracentrifugal method but I should like to indicate the terminology which we employ Since we are interested in studying the lipoproteins which are of low density the first step consists in separating them from the high density proteins simply by preparative ultracentrifugation There is no chemical procedure involved other than increasing the density of the solution by sodium chloride

* Supported in part by the U S Public Health Service and the Atomic Energy Commission

COMPOSITE PICTURE AS OBSERVED



RESOLUTION INTO UNKNOWN COMPONENTS

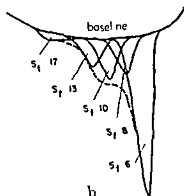


FIGURE 3 a Diagram illustrating a typical flotation pattern observed in the ultracentrifugal analysis of the low-density lipoproteins of human serum b Resolution of 2(a) into the lipoprotein components known to exist in human serum Reprinted from article by Gofman *et al* *J Gerontol* **6** *109* *(1951)*

to the identification and isolation of several independent lipoprotein components Thus the diagram of Figure 3 is resolvable into several lipoprotein species starting from S_f 6 and going up to S_f 17 Thus this composite picture with a sloping off of the left hand limb of the pattern is due to the algebraic addition of S_f 6 S_f 8 S_f 10 S_f 13 and S_f 17 patterns There are at least ten discrete species of lipoprotein molecules that we have been able to demonstrate and there are many more that we have not been able to resolve into discrete components as yet

This immediately points out that neither so-called alphalipoprotein nor so called betalipoprotein is a discrete entity Those mole

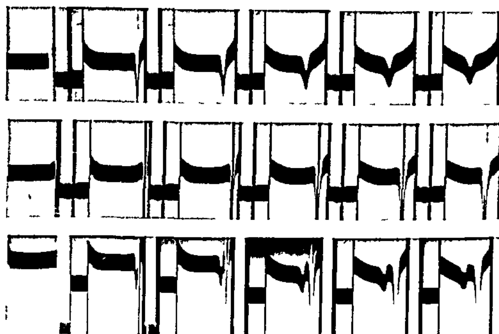


FIGURE 2 Upper pattern showing flotation of isolated S_f 13 molecules Middle pattern showing flotation of isolated S_f 6 molecules Lower pattern showing flotation of mixture of these two lipoproteins Reprinted from article by Gofman *et al J Gerontol 6 107 (1951)*

temperature viscosity and density then the rate at which a molecule moves is a physical constant for that molecule and is a perfectly adequate name for that molecule So that we can name molecules as S_f 13 or S_f 6 molecules This in essence tells us that the two lipoproteins with different flotation rates are different molecules They may both contain cholesterol cholesterol esters and fatty acids but they are different molecules

In order to determine the concentration of any species one draws in the base line which would be what one would see in the absence of lipoproteins and simply measures the area over the peak (see shaded area – Figure 1) From that area with a conversion factor one can directly calculate the concentration in mg percent of the particular species studied

Unfortunately in the study of serum one often has a more complex picture than that shown in Figure 2(c) One may see a complex diagram as shown in Figure 3 This type of pattern requires interpretation

In the course of the last year and a half the studies of serum done largely by my associates Lindgren and Nichols(1) have led

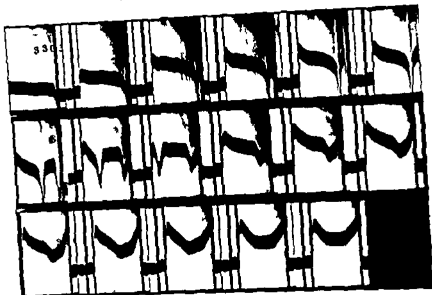


FIGURE 4 Ultracentrifugal flotation patterns of a total lipid and lipoprotein fraction obtained from a single individual. Frames from left to right (and top to bottom) were taken at 0 2 4 6 8 10 16 24 32 40 48 56 64 80 96 112 and 128 minutes after rotor attained full speed (59 780 rpm) Reprinted from article by Lindgren Elliot and Gofman. *J Phys Coll Chem* 55 84 (1951)

groups We have seen low and high concentrations of high density lipoproteins with any particular concentration of the low density group

In connection with atherosclerosis we have focused our attention on the low density group of molecules which would be represented by the first peak shown in Figure 4 The rabbit developing athero sclerosis has been studied in an effort to determine whether there is anything peculiar or new about lipid transport in the course of development of atherosclerosis as the result of cholesterol feeding

Figure 5 shows the sequence of events occurring in the rabbit fed cholesterol and developing atherosclerosis The rabbit initially shows a little peak which is due to a lipoprotein of about a million and a half molecular weight units The molecule is 30 percent cholesterol about 25 percent protein It is the major cholesterol bearing molecule in the rabbit in the normal state the rabbit having only about 40 or 50 mg percent of cholesterol in the serum If one feeds the rabbit cholesterol some two weeks later all one sees is an increase in the concentration of the same molecule as

cules in electrophoresis which would migrate as an alpha peak and those molecules which would migrate as a beta peak represent between 3 and N molecules in each case where N is some number the exact magnitude of which is not exactly known It is at least 10 for the so called beta peak in electrophoresis

Gutman Dr Gofman do your peaks necessarily represent homogeneous substances?

Gofman What do you mean by that? For the mixture of molecules?

Gutman No, I mean even for a single peak Does a single peak necessarily represent a single homogeneous substance?

Gofman *There is no criterion one can ever say is the absolute* criterion for homogeneity We can take a preparation of so called pure S_f 13 and ultracentrifuge it and from the diffusion constant one can say this molecule fulfills pretty well the criterion for being a homogeneous species But if one evolves a new technique for looking at something he may be able to resolve this into more than one species That is if one identifies a molecule and says he has ultracentrifugally a single molecule that does not mean that he has proved that by electrophoresis it will be a single molecule It may be two or three So all we can speak for is the ultracentrifugal homogeneity *But if we can say that we can see ten species then* we know there are at least ten Whether that may be twenty we cannot say but we know there are at least ten That is all I can say at the present time

Figure 4 gives the entire lipoprotein spectrum in a manner that is not easy to analyze but such that all the serum lipoproteins are shown If we raise the density of the serum to about 1 24 with a heavy water salt solution we get two groups of peaks Each of them is made up of several species The rapidly moving group peak is due to the low density lipoprotein molecules in the serum There are approximately ten *The slower group peak which later* shows itself to be at least a bifid peak represents the high density group of lipoproteins The latter peak probably corresponds to the so called alphalipoproteins the former corresponds to the so called betalipoproteins The concentrations of each group of lipoproteins are at least semi independent At the present time we are unable to see any consistent pattern of relationship between these two

appearance of appreciable levels of molecules of rates greater than 30 S_f units

Thus the whole picture of rabbit hypercholesteremia as it develops is first an increase in the molecule that was already there which may migrate from 5 to 12 S_f units then the appearance of additional molecules first with rates in the neighborhood of 10 to 20 S_f units primarily and then a high concentration of molecules all the way out to 30 S_f units and higher As the rabbit develops the hypercholesteremia he changes the molecules which carry that cholesterol to a predominant degree so that there are grossly different molecules present in rabbit serum at the end of such feeding as compared with what was there in the beginning

We have had the opportunity to autopsy about seventy five animals in the course of our experimental studies and find little correlation between the concentration of the molecule of less than 10 S_f units and the amount of atherosclerosis one measures at autopsy With increasing concentrations of these additional molecules there is a good rough correlation of degree of atherosclerosis with the concentration of molecules of this newer class which we might delineate at 10-30 S_f

It is true that the rabbit is developing a hypercholesteremia and we have no argument at all with the fact that high blood cholesterol is associated in the rabbit with the development of atherosclerosis but the primary form in which the additional cholesterol is carried is in these additional molecules There does not appear to be too much significance to the increase in concentration of the original lipoprotein molecule Rabbits that develop only an increase in this molecule with no new ancillary lipoprotein complexes do not show any significant atherosclerosis

Barr When the animals are developing the S_f 10 20 molecules are they at the same time increasing the amount of molecules of lower densities?

Gofman The total lipoprotein concentration (and cholesterol concentration) is increasing rather than simply a change in relative concentration

Barr Are the molecules of lower densities still increasing in amount?

Gofman Yes This is at 15 weeks There is a considerable increase from 6 weeks Some of the rabbits however do level out at a

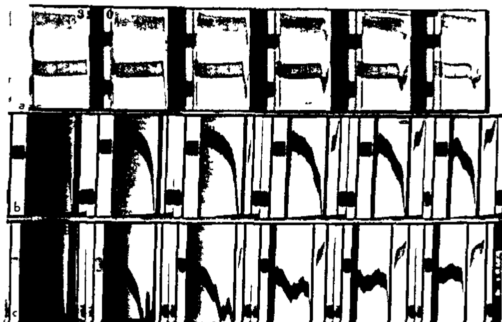


FIGURE 5 The progressive sequence of changes seen in the rabbit developing atherosclerosis as the result of cholesterol feeding

a The upper and lower ultracentrifugal patterns of the top film are those taken respectively at the outset of the experiment and two weeks later with a rise in the concentration of normally occurring lipoprotein in the latter

b Shows rabbit pattern at six weeks after feeding with appearance of high concentrations of lipoproteins above S_f10

c *Shows pattern after fifteen weeks feeding The extremely high concentration of* S_f 10 30 can be noted Reprinted from article by Gofman *Hypertension A Symposium* E T Bell Editor Minneapolis Minn Uni of Minnesota Press 1951 (p 383)

evidenced by a bigger area over that peak This means then that at least for a while the increment in cholesterol in rabbit serum is due wholly to an increase in concentration of a molecule that was already there in the first place But when six weeks pass one sees a very different situation in that although this peak (about 8 S_f units) is in the same position — and shows a further increase in concentration the rabbit has now developed several ancillary peaks representing molecules that are moving with rates faster than the 8 S_f units of the major peak — as a matter of fact moving with rates up to 30 S_f at about this point The additional molecules are lipoproteins containing cholesterol phospholipids and fatty acids though they contain less protein than the major molecule initially present In fifteen weeks a tremendous increase in concentration of the molecules in the S_f 10 30 class has developed plus the

appearance of appreciable levels of molecules of rates greater than 30 S_f units

Thus the whole picture of rabbit hypercholesteremia as it develops is first an increase in the molecule that was already there which may migrate from 5 to 12 S_f units then the appearance of additional molecules first with rates in the neighborhood of 10 to 20 S_f units primarily and then a high concentration of molecules all the way out to 30 S_f units and higher As the rabbit develops the hypercholesteremia he changes the molecules which carry that cholesterol to a predominant degree so that there are grossly different molecules present in rabbit serum at the end of such feeding as compared with what was there in the beginning

We have had the opportunity to autopsy about seventy five animals in the course of our experimental studies and find little correlation between the concentration of the molecule of less than 10 S_f units and the amount of atherosclerosis one measures at autopsy With increasing concentrations of these additional molecules there is a good rough correlation of degree of atherosclerosis with the concentration of molecules of this newer class which we might delineate at 10-30 S_f

It is true that the rabbit is developing a hypercholesteremia and we have no argument at all with the fact that high blood cholesterol is associated in the rabbit with the development of atherosclerosis but the primary form in which the additional cholesterol is carried is in these additional molecules There does not appear to be too much significance to the increase in concentration of the original lipoprotein molecule Rabbits that develop only an increase in this molecule with no new ancillary lipoprotein complexes do not show any significant atherosclerosis

Barr When the animals are developing the S_f 10 20 molecules are they at the same time increasing the amount of molecules of lower densities?

Gofman The total lipoprotein concentration (and cholesterol concentration) is increasing rather than simply a change in relative concentration

Barr Are the molecules of lower densities still increasing in amount?

Gofman Yes This is at 15 weeks There is a considerable increase from 6 weeks Some of the rabbits however do level out at a

certain value and show no further increase thereafter in spite of continued cholesterol feeding

These experiments were carried out in parallel with studies of human serum From Figure 6 it is apparent that the low density group of lipoproteins in human serum form patterns resembling those seen in various stages of rabbit atherosclerosis Figure 6(a) shows only one lipoprotein in human serum at any appreciable concentration This is a molecule which migrates with a rate of about 6 S_f units Throughout Figure 6(a) the S_f rates are marked on the successive frames, so one can immediately pick off any thing one wants If one is interested in knowing the S_f rate of a given peak one may read it directly off the grid for that frame

In the human we are somewhat hard pressed to establish direct correlations with extent of atherosclerosis or its rate of develop ment because we don t get to see the vessels and as we are all aware there are no clinical indices for appraising atherosclerosis in the living human in the absence of a clinical sequel There are certain correlations which suggest that one can get some informa tion on atherosclerosis from the ultracentrifugal lipid pattern

I am in full agreement with the concept that atherosclerosis is a focal disease in the human that if one has atherosclerosis in one vascular bed there is no guarantee that there will be atherosclerosis in other vascular beds The aorta may be affected and the other vessels relatively free and vice versa But I think there is still the possibility that whatever the focal factor is there may well be something in the blood associated with the process to cause it to develop at all in any vascular bed

Going on that basis we feel we have evidence with respect to the development of atherosclerosis Normal rabbits show only molecules of S_f 10 or less Rabbits fed cholesterol develop the addi tional molecules which migrate at rates greater than 10 Some human subjects show only the molecules below S_f 10 units and some show in addition as can be seen in Figure 6 varying con centrations of lipoprotein molecules out to about 80 or 100 S_f units and even higher

All of these molecules are shown by analysis to be lipoproteins which contain varying amounts of different lipids in association with protein As a broad picture we can say that the molecules of S_f rate less than 10 contain cholesterol cholesterol esters phos pholipids and protein We do not find by chemical analysis of

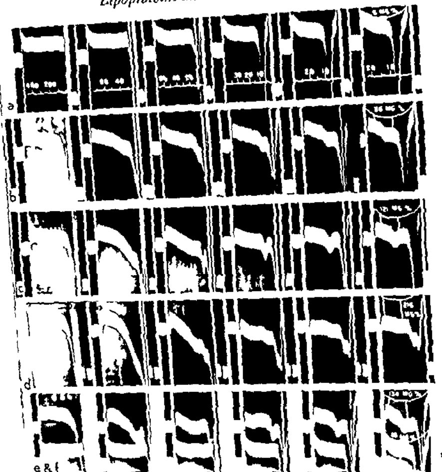


FIGURE 6 a Flotation pattern of low density lipoproteins from a normal 2? year old male showing exceedingly low level of S_f 10 20 molecules. Each frame is ruled for calculation of the S_f rate of any peak appearing in that frame. In this figure as well as in those below successive frames are at 0 6 12 22 30 and 38 minutes after full rotor speed of 52 640 rpm has been reached. Hence S_f rate markings may be used on corresponding frames in all patterns below.

b Flotation pattern of low density lipoproteins of a 65-year old male patient following myocardial infarction (well beyond acute phase)

c Flotation pattern of low density lipoproteins of a 36-year old male with hypothyroidism subsequent to surgical therapy for Graves disease

d Flotation pattern of low density lipoproteins of a 46-year old male with the nephrotic syndrome

e & f Analytic flotation pattern of two different samples run simultaneously. Upper pattern is that of a ?6-year old male studied three months *before* he experienced a myocardial infarction (Note Extremely high S_f 10-20 level. Six weeks beyond the acute infarction his level was found to be 29 mg percent) Lower pattern is that of a ?5-year old female demonstrating very low S_f 10-20 level. Reprinted from article by Gofman *et al* *J Geront* 6 *109 (1951)*

isolated species any evidence of neutral fat in those molecules The 13 S_f molecule also contains cholesterol cholesterol esters phospholipids, and *protein without any evidence of neutral fat* But as one gets out above 17 S_f units, neutral fat (or glyceryl ester) becomes a prominent part of the molecule In fact out at 50 100 S_f *units something in the order of 50 to 80 percent of the molecule* is composed of glyceryl ester, and less of the molecule is composed of cholesterol and cholesterol ester and less of the molecule is composed of protein The *striking charge about 17 S_f units* is the appearance of neutral fat as a part of the molecule This goes on all the way out to the chylomicron which on a relative scale is a molecule of S_f 40 000 units The chylomicron contains in the neighborhood of 5 percent cholesterol about 5 percent protein about 5 percent phospholipid with the rest being neutral fat One other interesting difference between the molecules as one goes out along this scale of classification is that the molecules become less dense the 40 S_f molecule is less dense than the S_f 6 probably because there is more fat and less protein in it

It has been established further that the percentage of cholesterol in the molecule in the form of cholesterol ester decreases progressively as one goes from S_f 6 to 10 13 and so on, until one reaches S_f 40 000 where essentially all of the cholesterol is in the free form whereas down in the S_f 6 molecule only about 20 percent of the cholesterol is free and the rest is esterified In the high density molecules which correspond to the alpha lipoproteins there is even less of the cholesterol that is free probably about 18 percent Thus these various species do not represent any agglomeration or polymerization in the ultracentrifuge They are molecules which differ in chemical composition one from another in addition to their differences in physical properties

With respect to the occurrence of these molecules in various human groups one can say this if one starts at the cradle (umbilical cord blood) and follows the changes through the group of normal prepuberal children the picture is almost wholly that of Figure 6(a) namely the presence of molecules of S_f 8 or less as the predominant species There may be small amounts of S_f 10 molecules and of *molecules that migrate faster than 10 units* but it is quite rare to find a young child with a level over 30 mg percent of molecules that migrate at rates greater than 10 or at least *in* the region from about 10 to about 30

I would like to focus attention on one particular class of these molecules the region S_f 10 to 20 units not because we have pre

sumed that this class would be involved in atherosclerosis but because the relationships in the rabbit suggested this group of molecules might be important in the human We feel that most likely other molecules may be involved but there are certain reasons why we haven t yet made extensive efforts to correlate them with disease For example one could say What about the 40 S_f molecule or the 50 S_f molecule?" One of the big reasons why this is a poor molecule to use or to test for correlation is that it is highly influenced in some individuals by the time after meals that one draws the blood sample and by the character of previous meals Since there may be threefold changes in the concentration of molecules for example between 30 and 100 S_f with relation to meals we find it difficult to use this as an index unless a rigid form of tolerance test is developed

But on the other hand as Dr Kendall mentioned earlier where the molecule is below 30 S_f between 10 and 30 — as a matter of fact right now I will speak only of 10 20 — there is very little influence of a single meal on these molecules and it does not depend on whether the blood is taken after fasting or after taking a meal

Wakerlin Are there any sex differences in these experiments? I am thinking of Dr Dock s observations on the coronaries

Gofman No on the basis of a limited study of about a hundred children we find no differences in terms of male infants versus female infants or male children versus female children before puberty

As can be seen in Figure 6 in the cross hatched areas we have measured the so called S_f 10 20 class The S_f 10 molecule itself which is one species is not included in this area only everything above 10 S_f The lower measuring limit is S_f 12 We are calling that S_f 10 20 with S_f 10 itself excluded We also have measurements on the S_f 10 itself which I will refer to later

A study of normal" human subjects of various age categories is illuminating In Figure 7 the percentage of individuals with S_f 10-20 levels below any given level is plotted for males From this figure one can compare 20 30 year old males with 50 60 year old males and determine what percentage of 20-30 year old males will have levels higher than a certain level as compared to the older group Thus approximately 25 percent of the young males fall above a level of 40 mg On the other hand approximately 55

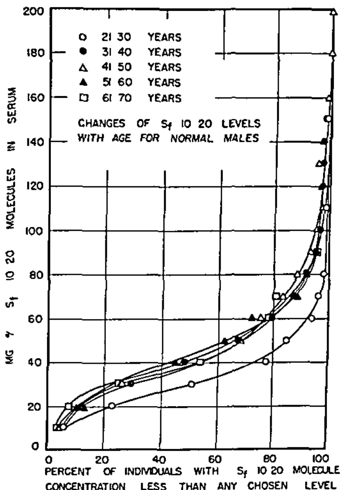


FIGURE 7 *Diagram illustrating the trend with age of blood levels of* S_f *10-20* lipoproteins in normal males 20 70 years of age

Notes (1) The lipoprotein levels reported here have been revised upwards as a result of calibration changes as compared with previous data reported in Circulation 2 161 178 1950 The revision in no way alters the *interpretations given in that reference*

(2) Data based on 977 cases

percent of the 50 60 year old males will have higher levels than this

The levels for the 20 30 year old male are higher than for the male child We feel this increase with age corresponds with the higher incidence of atherosclerosis with aging and with the comparative rarity of atherosclerosis in children

Dexter Approximately how many are in those groups?

Gofman I think the smallest group was about 50 Usually they consist of about 100 or more in each

The actual levels of mg percent are somewhat higher than our data in *Circulation* (2) indicated due to a revision of some calibrations However the distributions are essentially the same among the various populations

Figure 8 shows a similar curve for normal" females with a major difference between the 20-30 and the 50-60 groups indicat

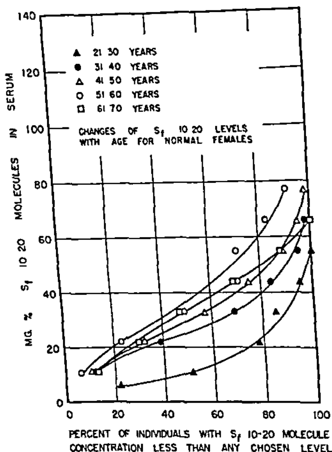


FIGURE 8 Diagram illustrating the trend with age of blood levels of S_f 10 20 lipoproteins in normal females 20-70 years of age Reprinted from articles by Gofman et al J Gerontol 6 11° (1951) and Gofman *Hypertension A Symposium* E T Bell Editor Minneapolis Minn Univ of Minnesota Press 1951 (p 386)

NOTE Data based on 309 cases

ing that fewer normal females at a young age will have a high level as compared with the older females In other words if you consider a level of 40 mg percent about 5 percent of normal females between 20 and 30 will be higher than that, whereas, 45 *percent of females* between 50 and 60 *will show higher levels* There are thus about nine times as many females between 50 and 60 who show high S_f levels We feel this is *consistent* with the rarity of atherosclerosis *in the young female and the fact that* differences in the occurrence of atherosclerosis in the two sexes become progressively obliterated with aging

When the 50 60 year old normal female is compared with the 50 60 year old normal male, these curves almost superimpose These curves were obtained in studies on several hundred cases

Summarizing these studies — children show the lowest levels the young females lower than young males and the young female lower than the older female and progressive obliteration with aging of the difference between sexes

As an index of atherosclerosis in the living we have resorted to the use of myocardial infarction survivals Myocardial infarction should give a good documentation of the disease and although certain myocardial infarctions occur superimposed on coronary diseases other than atherosclerosis and although coronary disease does not necessarily indicate atherosclerosis elsewhere in the body we feel that a group of men who have had a myocardial infarction on the average should have more coronary atherosclerosis than the so called normal population This of course will only be true on the average

Figure 9 shows some 203 males between the ages of 40 to 70 who survived a myocardial infarction compared with a composite curve of our so called normal males between 40 and 70 The small changes with aging between 40 70 years do not influence interpretations based upon the composite curve

Simms How long after the last attack?

Gofman We never took any of them unless there had been a minimum of six weeks after the infarction We chose not to study metabolic upsets of an infarction itself but rather to study steady state lipid metabolism *There are evidences that there are some* peculiarities associated with an acute attack Many cases were two years and some were ten years beyond infarction

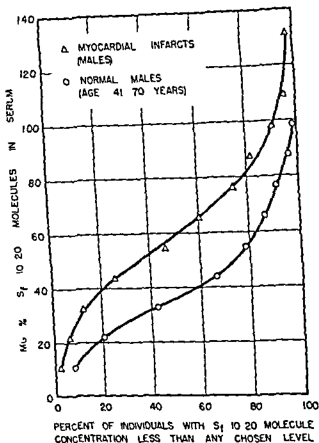


FIGURE 9 Diagram illustrating the higher levels of S_f 10-20 molecules in males with myocardial infarction than in normal males of corresponding ages Reprinted from articles by Gofman *et al* *J Gerontol* 6 112 (1951) and Gofman *Hypertension A Symposium* F T Bell, Editor Minneapolis Minn Uni of Minnesota Press 1951 (p 386)

Notes (1) All patients with infarction were at least six weeks beyond infarction date

(2) Data based on 203 myocardial infarcts and 241 normal males

Kellner You say that on the average the group with myocardial infarction will have more total atherosclerosis than a group of comparable age without myocardial infarction Is there any quantitative evidence for that other than impressions from the autopsy table?

Gofman I do not believe we have any justification based upon quantitative evidence that there will be more total atherosclerosis

in a group of patients with myocardial infarction However I believe the evidence is good that there will be more coronary atherosclerosis, on the average, *in such patients than in a group* of individuals who have not had an infarct Do you have some evidence to the contrary?

Kellner No My own impression from autopsies of individuals with myocardial infarction is that they have more coronary athero sclerosis but I am not sure as far as total atherosclerosis is concerned

Wilens Every experienced pathologist is aware that a young hypertensive male may die of myocardial infarction as the result of a few small atheromatous plaques that occlude a coronary artery without having any significant atherosclerotic change in any other vessel Often this is due to an unfortunate distribution of the coronary vessels with the occluded one supplying an unusually large area of the left ventricular musculature The implication of this observation is that the occurrence of myocardial infarction may give an entirely false clinical impression of the extent of the athero sclerotic process in any one person

Gofman Yes I certainly agree with that That is why I said at the outset one cannot quantitate the total degree of atherosclerosis *from what is present in the coronary artery and one cannot say* further that a myocardial infarct represents any evidence of total atherosclerosis

Grimson It occurs to me that there must be methods other than study of the coronary arteries by which extent of arteriosclerosis could be objectively determined One would be study of amputation specimens from people who have peripheral vascular obstructive disease You certainly can always confirm the normal circulation *and can estimate diminished circulation Diagnosis would be ac* curate and study of normal and diseased groups could be accom plished with little or no overlap

Gofman Studies on peripheral arteriosclerosis are being carried out but we do not have sufficient cases to make definitive statements

Wakerlin Wouldn t funduscopic examination be a better method for trying to separate your so called normal normals from ab *normal* normals?

Gofman I am not sure that is the case I have heard of disagree ment among ophthalmologists as to whether they see atherosclerosis in funduscopic examination

Dock There is no happy way to solve the dilemma that we are confronted with but I think Dr Gofman is reasonable in assuming that a population that has had a myocardial infarction has a greater tendency to coronary atherosclerosis than a population that is awaiting its first myocardial infarction In *Circulation* in the last year Dry(34) gives figures on the incidence of various degrees of atherosclerosis in autopsies in Rochester Minnesota In this article 3 plus coronary sclerosis indicates almost complete obstruction of one branch The shape of the curve in population with aging is quite different in males than it is in females They both level off with about 65 percent of the population having 3 plus coronary disease at 60 years in the male and 70 in the female Two thirds of the people will have 3 plus atherosclerosis in a major branch of the coronary artery The age curves are quite different in males than they are in females in those two things and agree with the shape of the curves Dr Gofman has shown

Gofman As I recall the White Edwards and Dry paper(3) they indicated an increase up through 50 to 60 and then a tapering off Is that recollection correct Dr Dock?

Dock When you examine the curves it appears that with 3 plus disease in one coronary artery the death rate will equal the rate at which such lesions are developing

Kellner Again they were measuring coronary atherosclerosis and not total atherosclerosis

Gofman Figure 10 shows a comparison of 27 females who have had a myocardial infarction with normal females in the same age group again showing a considerable spread Only 35 percent of normal females fall above the 40 mg level whereas about 80 percent of the coronary females are above this level

Figure 11 shows a comparison of 63 males and females who have had clinical angina pectoris without having had a frank myocardial infarction Blumgart and Schlesinger(5) have shown that the majority of such patients have extensive coronary atherosclerosis We have found that the patients who have the symptoms of angina pectoris have much higher levels of S_f 10 20 molecules than do the normals About 20 percent of the normals in the older age group are above levels of 60 mg percent whereas some of the patients with myocardial infarction are below this level This apparent discrepancy results from the probability that some of the normals have atherosclerosis although they have had no clinical episode I think

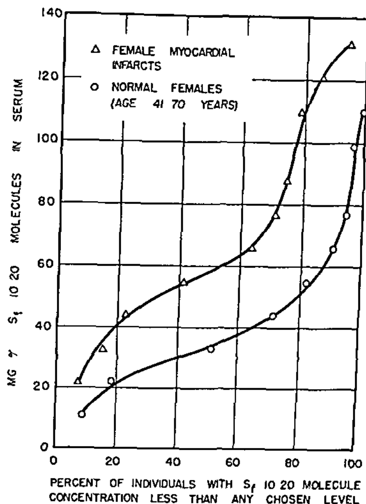


FIGURE 10 Diagram illustrating the higher S_f 10 20 levels in females with myocardial infarction than in normal females of corresponding ages Reprinted from articles by Gofman *et al* *J Gerontol* 6 113 (1951) and Gofman *Hypertension A Symposium* E T Bell Minneapolis Minn Uni of Minnesota Press 1951 (p 356)

Notes (1) All patients with infarction were at least six weeks beyond infarction date

(2) Data based on 27 female myocardial infarcts and 139 normal females

there may be a variety of explanations for the low values obtained with occasional patients with angina or myocardial infarctions Some patients may get into trouble at lower levels of these molecules when local factors favor the development of plaques Others have a non atherosclerotic basis for their disease

Figure 12 is a comparison of 95 patients who have diastolic hypertension and who have had a myocardial infarction or who

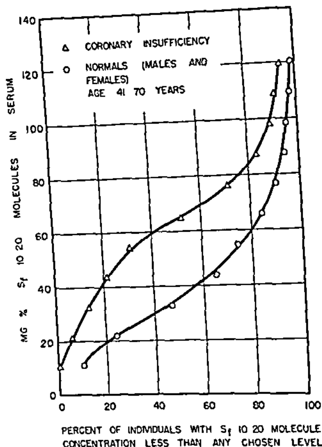


FIGURE 11 Diagram illustrating the higher S_f 10-20 levels in patients with coronary insufficiency (as manifested by angina pectoris) than in normals Reprinted from articles by Gofman *et al* *J Gerontol* 6 113 (1951) and Gofman *Hypertension, A Symposium* E T Bell Editor Minneapolis Minn. Uni of Minnesota Press 1951 (p 387)

Notes (1) No significant differences were found between the males and females in these groups so the plots are composite for both sexes

(2) Data based on 63 patients with coronary insufficiency and 380 normals

have angina pectoris or both compared with 139 patients who have hypertension with a diastolic pressure above 100 mm Hg but who have not had any overt evidence of coronary artery dis ease It is interesting to note that there is a considerable difference in S_f levels Patients with hypertension complicated by coronary artery disease have a much higher frequency of high levels than

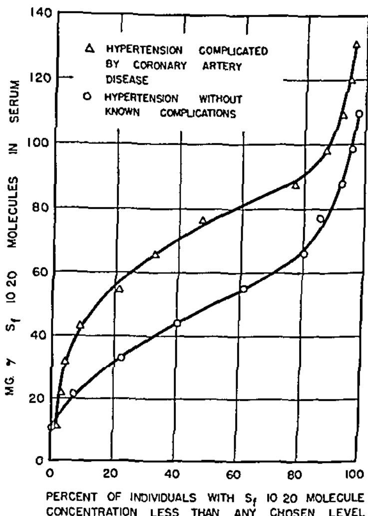


FIGURE 12 Diagram illustrating the higher S_f *10 20* lipoprotein levels *in hyper* tensives complicated by coronary artery disease than in hypertensives without such known complications Reprinted from articles by Gofman *et al J Gerontol 6 114 (1951)* and Gofman *Hypertension A Symposium* E T Bell Editor Minneapolis Minn Uni of Minnesota Press 1951 (p 387)

Note Based on 95 hypertensives with known coronary artery disease and 139 hypertensives without known coronary disease (Age group 41 70 years)

do those who show no evident coronary disease The hypertensives as a group are somewhat elevated above the normals The difference however is not as striking as that between the hypertensives with out coronary disease and the hypertensives who do have coronary disease

Wilens Have you evaluated the role of heart failure in your cases of coronary artery disease? Is it not possible that the changes in the character of the blood lipid might be associated with changes in

the blood resulting from heart failure rather than from coronary artery disease *per se*?

Gofman These are all ambulatory coronaries Some of them probably have had some degree of heart failure but no patients were included when they showed obvious heart failure Probably 90 percent of our coronary cases have no heart failure

Wakerlin Are some taking digitalis regularly and others not?

Gofman Some are I would say at least 80 percent are not

Wakerlin Might it not be worth while to divide those two groups and see if there is any further correlation?

Gofman In the initial studies we saw no significant difference between such groups

Gutman Dr Gofman is the age distribution about the same?

Gofman These are both between 40 and 60

Gutman Could you indicate where the apparently normal population would be?

Gofman They would be a little below the curve for hypertensives alone (See Figure 7)

Gutman No more than that?

Gofman Not much difference We cannot compare hypertensives as a group with normals if we include those hypertensives with coronary disease On the other hand one cannot discard hypertensives who have a coronary when studying the effect of hypertension on atherosclerosis

A similar study on a smaller series has been done with diabetics We have compared 56 diabetics who have no overt evidence of vascular complications with 15 cases complicated by coronary artery disease or diastolic hypertension or both There is a significant difference in S_f levels in those diabetics who have vascular disease as compared with those diabetics not yet having manifested vascular disease

With respect to both the diabetics and the hypertensives I think there is agreement that a fair number of diabetics and hypertensives go along without developing excessive atherosclerosis This is consistent with the generally accepted idea that as an overall group diabetics and hypertensives do develop more atherosclerosis than the overall population

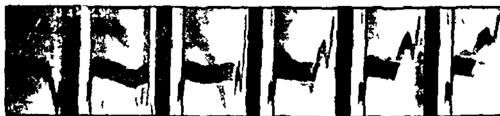


FIGURE 13 Upper pattern demonstrates extremely high S_f 10 20 concentration in a patient with myxedema Note the low level of the S_f 6 lipoprotein Reprinted from article by Gofman *Hypertension A Symposium* E T Bell Editor Minneapolis Minn Univ of Minnesota Press 1951 (p 388)

We have studied several diseases that presumably predispose to atherosclerosis Figure 13 (upper) is from a patient with myxedema I should like to point out that this first peak which is the S_f 6 peak is actually lower than one sees in most normals In other words even though this myxedematous patient has an elevated serum cholesterol there is *not* an elevation in all the cholesterol fractions Certain molecules such as the S_f 6 molecule are actually lower Evidence of this sort makes us feel that one should not include all the cholesterol containing molecules in the study of atherosclerosis *per se* since such molecules as the S_f 6 may be lower in disease groups where one suspects more than the usual atherosclerosis than in normals The hypercholesteremia in this hypothyroid patient is *largely in the form of the S_f 10 20 group and in the form of mole* cules with greater rates than S_f 20 In this patient the S_f 10 20 level is of the order of 300 mg percent which is higher than 96 percent of all patients with coronaries that we have observed

Barr Is that generic or in one individual?

Gofman This is one individual In eight cases of myxedema either primary myxedema without known cause or surgical myxedema from operated Graves disease this pattern was almost diagnostic I have yet to see a pattern in a frank myxedema that is significantly different from this one

In Figure 14 is the pattern of a case of xanthoma tuberosum There is little doubt that people with this disease have more atherosclerosis than the normal population Again I should like to point out that the S_f 6 class in this patient with xanthoma tubero sum, even with a cholesterol in the neighborhood of *some 700 mg* percent, is not too elevated In fact the S_f 6 is as low in many people who have low S_f 10 20 levels However the S_f 10 20 in this patient



FIGURE 14 Lower pattern demonstrates extremely high S_f 10-20 concentration in a patient with xanthoma tuberosum Note the low l vel of the S_f 6 lipoprotein

with xanthoma tuberosum is very high This has been uniform in all the cases of xanthoma tuberosum we have studied As a group they show the highest levels of molecules in the S_f 10 20 class that we have seen This again is consistent with the possible relation of these molecules to atherosclerosis

Studies on patients with varying blood levels of cholesterol from 120 mg percent to 300 or 500 mg percent show that one cannot predict from the cholesterol level in an individual case what the S_f 10-20 level will be At a cholesterol of 250 the level of these molecules can be down at about the limits of resolution or the levels of these molecules can be 100 mg percent or higher On the other hand for groups of patients as the cholesterol level rises there is a general trend toward increase in the level of S_f 10 20 molecules Despite this there is only a low correlation ($r = 0.4$)

Several approaches are being followed from the point of view of establishing significance of these molecules with respect to athero sclerosis One involves experimental studies in which we are trying to isolate these individual fractions ultracentrifugally and to deter mine with labels whether there is selective incorporation into the plaques or selective retention in arteries once deposited Another approach is to determine whether these molecules are of any prognostic value We are studying several classes primarily nor mals over a period of years in an effort to determine whether after having measured the levels of the molecules of these people we can then relate this to their subsequent course This approach cannot prove that these molecules are the cause of arteriosclerosis In addition in people coming to autopsy after myocardial infarc tion it will be possible to ascertain the degree of atherosclerosis throughout the body in relation to the levels of these particular molecules

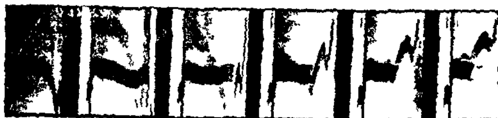


FIGURE 13. Upper pattern demonstrates extremely high S_f 10-20 concentration in a patient with myxedema. Note the low level of the S_f 6 lipoprotein. Reprinted from article by Gofman. *Hypertension. A Symposium*. E. T. Bell, Editor. Minneapolis, Minn.: Univ. of Minnesota Press, 1951 (p. 388).

We have studied several diseases that presumably predispose to atherosclerosis. Figure 13 (upper) is from a patient with myxedema. I should like to point out that this first peak, which is the S_f 6 peak, is actually lower than one sees in most normals. In other words, even though this myxedematous patient has an elevated serum cholesterol, there is *not* an elevation in all the cholesterol fractions. Certain molecules, such as the S_f 6 molecule, are actually lower. Evidence of this sort makes us feel that one should not include all the cholesterol-containing molecules in the study of atherosclerosis *per se*, since such molecules as the S_f 6 may be lower in disease groups where one suspects more than the usual atherosclerosis than in normals. The hypercholesteremia in this hypothyroid patient is largely in the form of the S_f 10-20 group and in the form of molecules with greater rates than S_f 20. *In this patient the* S_f 10-20 level is of the order of 300 mg. percent, which is higher than 96 percent of all patients with coronaries that we have observed.

Barr: Is that generic or in one individual?

Gofman: This is one individual. In eight cases of myxedema, either primary myxedema without known cause or surgical myxedema from operated Graves' disease, this pattern was almost diagnostic. I have yet to see a pattern in a frank myxedema that is significantly different from this one.

In Figure 14 is the pattern of a case of xanthoma tuberosum. There is little doubt that people with this disease have more atherosclerosis than the normal population. Again I should like to point out that the S_f 6 class in this patient with xanthoma tuberosum, even with a cholesterol in the neighborhood of some 700 mg. percent, is not too elevated. In fact, the S_f 6 is as low in many people who have low S_f 10-20 levels. However, the S_f 10-20 in this patient

infarct and those who do not However that does not imply that *people who go along* with massive atherosclerosis without precipitating a clinical episode do not have *the disease*

Katz If you study a group of 300 patients with atherosclerosis and a group without it what would be your prediction about the frequency of coronary occlusions and infarctions in the two groups?

Gofman My prediction would be that there would be more infarctions in those with atherosclerosis

Katz In other words since myocardial infarctions are more frequent in people with atherosclerosis any plasma analytical test that predicts the presence of atherosclerosis is extremely valuable — particularly if ways are found to remove the lipid elements participating in atherogenesis

Gofman I believe so

Dock Are there observations on changes in these fractions in patients who alter their way of life after a myocardial infarction?

Gofman Yes

We know that the large proportion of patients who go on a diet *restricted* in fats and cholesterol show reduction in the level of the S_f 10 20 class of molecules If one then allows these people *to add vegetable* oils in large amount to their diet with no cholesterol added their S_f 10 20 levels will rise again In other words it seems as though fat ingestion is involved in this as well as cholesterol

This brings up an interesting point on acute coronaries One man normal with respect to history and physical examination was studied for some six months because his S_f 10 20 level was 131 *mg* percent About two and a half months ago he had his *first coronary* occlusion at the age of 38 He was in a hospital for *six weeks* and was placed on a low fat low cholesterol diet during that period At the end of seven weeks we studied him again and his level at *the end of seven weeks following his* coronary was 29 *mg percent* This possibly may explain some of our low level myocardial in*farcts in that* many who *do have* this occurrence necessarily go on restricted *diets* Certainly during hospitalization they are on a restricted diet *and many* of them remain on it afterwards If we had seen this case *for the first time* after his infarct we would have seen a man *with a level* of 29 mg percent However we know

A relative comparison of S_f ratios with cholesterol phospholipid ratios indicates that molecules from S_f 3 to 100 have weight ratios of cholesterol to phospholipid in the neighborhood of one Molecules of S_f 10 20 classes on the basis of our most recent determinations may be as high as 1 3 However, the high density lipoproteins probably corresponding to the alphalipoproteins that Dr Barr mentioned have a weight ratio of cholesterol to phospholipid in the neighborhood of one half The overall serum cholesterol phospholipid ratio is therefore determined more by the relative amount of high density lipoproteins compared to low density lipoproteins than it is by the distribution of cholesterol among the group from 3 to 100 S_f

Another interesting group are the 4 or 5 patients with biliary obstruction whom we have had the opportunity to examine Several of these patients have blood cholesterol levels of about 1200 mg together with a fantastic elevation of the S_f 6 molecules Since they have low levels of the S_f 10 20 class we predict that they should be relatively free of atherosclerosis On the other hand some of these patients show appreciable concentrations of S_f 10 20 class molecules but at much lower levels than would correspond with their 1200 mg percent of cholesterol In all of these cases there is a sharp cutoff above S_f 20

Goldblatt Dr Lansing how would you classify the following two hypothetical cases? One is a patient with a 4 plus patch of *atherosclerosis in one coronary artery with a thrombus at the site* of the sclerosis and massive infarction of the heart like some of Dr Gofman s patients and the other patient has plus minus arterio *sclerosis but it involves all the branches of the coronary artery* and is also in the same degree in the aorta and all arteries of the body How are you going to compare those two cases in so far as *the degree of arteriosclerosis is concerned? I am not certain it can* be done

Lansing I think there is a genuine chance factor involved here We have all seen rather minor lesions in the coronary that bring the individual to the autopsy table Also rather massive lesions involving large accumulations of fat are seen in patients dying from causes other than coronary disease It is difficult to classify the disease on a quantitative basis

Gofman I do feel that in taking group averages one can detect a difference in the coronary arteries among those who have an

Gofman We have studies on the effects of male hormone and female hormone but they are not far enough along to make any definitive statement

I do think that the dietary approach is one way of altering the S_f 10-20 molecules However as a fundamental element I dont think that this difference between people is a dietary matter certainly not to the largest extent We can find no correlation with the usual range of American diets

Secondly one interesting group are the young females who become pregnant We studied about 40 females during pregnancy During the second and third trimester over 75 percent of these females developed high levels of the molecules of the S_f 10 20 class They may develop levels of 60 to 100 mg percent which puts them up in the top range of the coronary class Some of these females were studied six weeks several months or a year after delivery and were back to normal levels

Shorr Are any of these pregnant women receiving thyroid?

Gofman Some are and some are not Some who are receiving thyroid still show high levels The effect of thyroid extract is not easily predictable With some hypothyroids who are receiving thyroid we have seen drops in the levels of these molecules We have studied 20 patients who were schizophrenics whose thyroid status was not determined directly and who for another reason were receiving from 3 to 10 grains of thyroid per day Some of them showed profound drops in all the molecules S_8 up to S_f 20 on thyroid extract and some did not show these drops In some patients on thyroid you find drops in S_8 without big drops in S_f 10 20 The effect is not uniform

We are studying a group of individuals who are euthyroid clinically and from basal studies These patients are being treated with small doses of thyroid to see whether we can influence the S_f 10 20 molecules without dietary manipulation The results are not striking A few have shown significant drops but the majority of euthyroid patients on doses of ½ Gr to 1½ Gr of thyroid show no significant changes

Shorr Doses of this order of magnitude should not be expected to have any appreciable effect inasmuch as they would merely lead to a proportionate readjustment of the activity of the patient s own thyroid

that this man had previously carried for at least six months a level of about 130 mg percent

Barr Was his total cholesterol level very high?

Gofman His total cholesterol level was in the neighborhood of 320 mg percent

Barr Did it become very much lower?

Gofman I have no data on his total cholesterol level when the S_f 10 20 level fell In patients on dietary restriction the total cholesterol level will drop significantly in most cases, but only insignificantly in others The correlation between the percentage drop in total cholesterol and the drop in S_f 10 20 is poor Some patients show larger drops in total cholesterol than they will in S_f 10 20

Dock You have no collections from the blood of Okinawa citizens to compare with the 50 to 60 year old Americans

Gofman General Sams promised us 200 bloods from Japanese that are living on 20 gm of fat and practically no cholesterol

Simms Do you have controls on a normal American diet in the same race?

Gofman We do not have those It might be well to study Japanese who live on higher dietary fat and cholesterol intakes

Shorr Have you broken down the data as to the changes observed with increasing age on the basis of the weight of the subjects?

Gofman Yes we have taken people of a given age category and plotted S_f 10 20 levels and the function of weight A correlation between weight and these molecules is practically nonexistent The long lean individuals can have much higher levels than those that are 30 pounds overweight There is practically no correlation with obesity

Shorr A second question have you broken down the figures for women in relation to the menopause?

Gofman Not directly on that basis

Shorr Have you any patients who have been receiving estro genic treatment?

Gofman We have studies on the effects of male hormone and female hormone but they are not far enough along to make any definitive statement

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Shorr Doses of this order of magnitude should not be expected to have any appreciable effect inasmuch as they would merely lead to a proportionate readjustment of the activity of the patient s own thyroid

Gofman Yes Ten grams of thyroid in the schizophrenics showed several who were not responsive at all

Stamler How about people with Cushing s syndrome or patients on cortisone or ACTH?

Gofman We studied a small series of patients receiving cortisone and a series of patients receiving ACTH In both groups in spite of the fact that there is clinical evidence that they have responded to cortisone or to ACTH they showed insignificant changes in the levels of these molecules in short term experiments of several weeks

In the experimental rabbit cortisone and ACTH produced pro found effects on the level of these molecules the effects not being uniform from animal to animal With cortisone one may see in rabbits that have had normal patterns never having been fed cholesterol fantastic rises in all the molecules of the S_f 10 20 and higher classes, and then a recession to normal However the effect of cortisone in the human with the doses employed has not been striking

In the rabbit one sees these molecules with cholesterol feeding but this does not imply that the rabbit can only get these molecules from cholesterol feeding We have given a series of rabbits carbon tetrachloride by injection in an effort to produce liver injury – of course carbon tetrachloride is an agent that will also produce damage to other organs besides the liver – and one can reproduce the entire picture seen in the rabbit with cholesterol feeding alone Evidence of this kind suggested to us the sort of thing that Dr Kendall mentioned – that all these molecules may be part of the lipid trans port mechanism When one sees a rise it does not necessarily mean that new molecules are being formed there may be an abnormal situation which involves either putting too many in the blood or taking too few out We believe that in the carbon tetrachloride injected animals where there has been no cholesterol feeding some thing has blocked utilization

Wilens Do the rabbits with large lipid molecules following car bon tetrachloride injection develop atherosclerosis?

Gofman The group on carbon tetrachloride were not followed sufficiently long with high levels to say definitely that they do

Goldring Dr Gofman can we assume from your charts no over lap of data between so called control and coronary patients?

Gofman Let us consider a level of 60 mg percent We can merely say that 50 percent of patients who have survived a myocardial infarction are above that level and 50 percent are below For so called normals about 17 percent are above that level and 83 percent below So that 17 percent of normals have higher levels than 50 percent of coronaries This we feel is consistent with the fact that many of these normals have atherosclerosis

Wakerlin Have you attempted to see whether in normals you could get anything from the standpoint of heredity? In other words this 17 percent showing the Sf 10 20 molecules might possibly be the offspring of parents both of whom have had a death due to cardiovascular disease particularly cardiac infarction

Gofman We know there have been high levels in offspring of parents who have lived from 80 to 90 We do not have a statistically significant study We are in the process of studying all the members of about 200 families to see whether, with parents who have high levels there is a higher than normal incidence in children

We have an interesting illustration in twins two women identical twins in their fifties both of whom have levels of 140 mg percent That would place them up in the 1 percent bracket for normal women We also have two children identical twins at the age of 7 and they both have levels in the neighborhood of 40 mg percent That is a rather low level but high for children

Katz Have you not stated that lipotropic factors exert a negligible effect on Sf 10-20 levels?

Gofman We have seen no effects of the lipotropic agents including inositol choline and methionine

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THE REGULATION OF FAT DEPOSITION BY THE LIPFANOGENS AND ANTILIPFANOGEN*

HENRY S SIMMS

Department of Pathology, College of Physicians and Surgeons Columbia University

WHAT I HAVE to say involves somewhat different terminology from what you have been listening to today That does not mean that I am going to talk about a different subject or about different substances It may eventually be found that we are working with the same substances and that these are merely being studied in a different manner in our respective laboratories

The word *Lip fan o gen(s)* comes from the Greek lipos for fat, phaneros for visible, and gen to produce The definition is that *the lipfanogens represent a special group of lipid substances found in blood plasma which when in a free state are taken up by living cells and converted into visible fat*

Our work involves tissue culture studies of adult chicken aorta fibroblasts The cultures are used for observing the mechanism of fat deposition and particularly for studying the agents in blood plasma which cause or prevent the deposition of fat as observed in these tissue cultures

Those of you who are familiar with tissue cultures know that the cells become filled with granules which consist mainly of neutral fat and are stainable with Scarlet Red These granules are produced by lipfanogens that are present in the blood plasma or the blood serum that is used in the culture medium It is possible to obtain cultures free from fat by a special technique that involves washing with serum ultrafiltrate (which does not contain the lipfanogens but does contain a stimulating agent)

The lipfanogens are always present in blood plasma and in almost all tissues They are heat stable unfortunately for our work They represent two main groups of substances, one group being ether soluble and the other ether insoluble It is conceivable that the ether insoluble

* This investigation has been aided by grants from the National Heart Institute of the National Institutes of Health of the United States Public Health Service the Josiah Macy Jr Foundation the Albert and Mary Lasker Foundation the New York Heart Association and the Life Insurance Medical Research Fund

lipfanogens may correspond with the lipoproteins that we have been hearing about today In other words when we extract with ether we remove about 50 percent of the lipfanogen activity

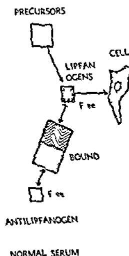


FIGURE 15 Relationship between fat regulating agents in normal serum

In this chart (Figure 15) the free lipfanogens are represented by the small square and bound lipfanogens by the larger square below it These are bound to antilipfanogen

The *antilipfanogen* is a material present in blood plasma which can be destroyed by heating at 80 C or higher The antilipfanogen is present in the serum albumin fraction (Cohn s Fraction V) and further fractionation of that material gives higher activity The antilipfanogen combines with the lipfanogens forming a complex which is inactive in that it does not cause fat deposition Fat deposition is caused by the free uncombined lipfanogens Thus, in normal serum we have lipfanogens both free and bound with antilipfanogen We have antilipfanogen free and bound with lipfanogens

If we take a sample of serum and dilute it divide it into three portions and then heat one portion we kill the antilipfanogen Then the extent of fat granule formation which that portion produces in a culture corresponds to the total amount of lipfanogens (represented by the small square plus the larger square)

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FIGURE 16 Chicken innominate artery segment incubated two days in serum ultra filtrate then four days in a medium containing lipfanogens x100 Stained with Scarlet Red Reprinted from article by Simms and Stillman Arch Path 23 336 1937)

Simms No no other observed changes except the fat deposition

Stamler Are there any control preparations of vessels treated similarly but lacking lipfanogens in the nutrient solution which remain free of intimal fat accumulation?

Simms In each case alternate segments were used as controls and whenever any fat was found in a control we discarded the whole experiment

Kellner If that section were stained with H & E would those cells appear to be normal viable cells or would they appear necrotic?

Simms They are normal cells at this stage If kept longer they become necrotic Incidentally the fat is not in the cells In our tissue cultures fat appears as granules in the cells However in the artery segments it is outside the cells

In another culture treated with a portion of unheated serum we get fat deposition corresponding to the free lipfanogens only (small square) From these values we can compute the amount of lip fanogens, the amount of antilipfanogen and the amount combined The combination of antilipfanogen with the lipfanogens follows an equilibrium equation, and that equation makes it possible to compute the concentrations of these materials

Serum also contains precursor material which, on incubation is converted into lipfanogens (apparently an enzymatic reaction) The third portion of the diluted sample is therefore incubated at 37° for 3 days then heated at 90° C to kill the enzyme The increase in the amount of lipfanogens corresponds to the concentration of precursors originally present in that sample of plasma On a 1 ml sample of serum we can determine the amounts of these constituents (expressed in arbitrary units)

What is the evidence that these agents the lipfanogens and antilipfanogen are involved in causing or preventing the fat deposition in atherosclerosis? In the first place the amount of free lipfanogens in those species such as the dog and horse that normally have low atherosclerosis is considerably lower than in humans In chickens where atherosclerosis is prevalent the free lipfanogens are high Secondly we can take segments of arteries from either chickens or humans treat the segments with lipfanogens *in vitro* and obtain deposits of fat suggestive of atherosclerosis

Figure 16 shows a segment of innominate artery of the chicken treated *in vitro* with lipfanogens and then stained with Scarlet Red after sectioning The deposition of fat here though not similar to that in humans is very suggestive of that occurring *in vivo* in chickens

Katz How long did this treatment continue?

Simms The chicken artery segment was incubated for two days in serum ultrafiltrate then for four days in a medium containing lipfanogens With human material there is a much greater resistance to the deposition of fat In ten days (which is about as long as we can keep the tissue alive) we obtained only a slight deposition of fat in the intima suggestive of an early lesion but not nearly as conspicuous as the depositions in the chicken artery

Stamler I would presume that in an *in vitro* culture there is no fibrosis or thickening of the intima secondary to lipid deposition?

Kellner I wonder whether this material has been made by the tissue itself from constituents

Simms No our evidence is against that Soaps of fatty acids of different molecular weights produce fat deposits of different solubilities With the long chain fatty acids the solubility is considerably different from that for the short chain fatty acids In other words the soaps act as lipfanogens and we can regulate the type of fat deposited by using different soaps Hence we know that material brought in from the outside is deposited

Figure 17 is a photograph of a tissue culture to illustrate the type of fat granules that are normally observed The cells are adult chicken aorta fibroblasts with conspicuous fat granules throughout the cytoplasm

Figure 18 represents three cultures treated with three portions of a sample of serum The first portion was unheated the second was heated at 90 C the last one was incubated for 3 days and then heated

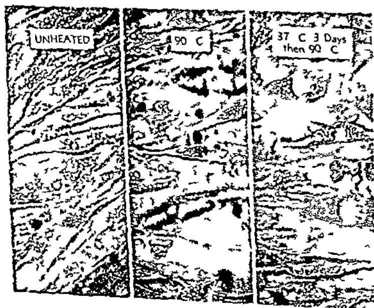


Figure 18 Fat granule production by three portions of a sample of human serum (coronary disease) One portion was unheated one heated at 90 C and one incubated before heating

Gofman Is there any lipid stain in the cells that line the intima?

Simms No The same was true in the human artery the small fat deposits were outside the cells

Wilens Is the fat deposited in the vessels anisotropic?

Simms No The same answer applies to the tissue cultures In general we can observe nothing under polarized light There was one case however of a sample of hypercholesteremic rabbit serum that was tested years ago in which we found conspicuous anisotropic droplets in one of the cultures We have not been able to reproduce it since There is nothing to indicate whether these fat deposits include cholesterol or not I am inclined to believe that cholesterol does enter the cells in these fat granules but I have no proof of it at present

Kellner Dr Simms is there an increase in the total amount of lipid in the tissue or is it simply lipid becoming visible?

Simms We have never analyzed for it



FIGURE 17 Example of fat granules in a culture of adult chicken aorta fibroblasts No stain

with a standard volume of serum at a standard dilution and in a standard size flask at a standard temperature and a standard pH

A study was made of the concentration of these materials in serum from normal individuals and from patients with different diseases On the left side of Figure 19 we have the serum lipids

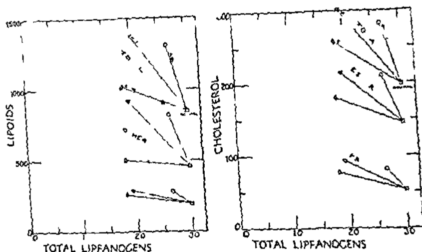


FIGURE 19 Relationship between the total lipfanogens and the lipid analyses in normal and hypercholesteremic sera

the phospholipids the total lipids and others On the right is the cholesterol free ester and total In each case the squares represent normal individuals When the lipids rise above normal the total lipfanogens go down The greatest effect is observed with diabetes nephrotic syndrome comes next and the least effect is with coronary disease These values are the total lipfanogens In other words in conditions in which there is hypercholesteremia conditions associated with atherosclerosis we find that along with the high lipids there is a drop in total lipfanogens This is in contrast with the *free lipfanogens* which are *higher* in hypercholesterolemia

The total antilipfanogen also drops in these diseases in a similar manner (Figure 20)

The ratio between the two indicates that the lipfanogens drop less than the antilipfanogen (Figure 21) It can be seen that the ratio of total lipfanogens divided by total antilipfanogen is roughly proportioned to the lipid concentration

You will see that the cells in the first culture (where antilipfanogen was present) have much less fat than in the second culture This represents only the free lipfanogens (the remaining lipfanogens being bound with antilipfanogen) In the second culture we are dealing with the total lipfanogens present in the serum In the third culture we have the original lipfanogens plus additional lipfanogens produced from the precursor

This is a representative test run with every sample of serum We have an arbitrary scale of rating which has been calibrated against the actual volume of fat in the cells and also calibrated against relative concentration of lipfanogens In estimating the amount of fat deposition we observe a much larger microscopic field than shown here We are able from our arbitrary scale of rating to compute the lipfanogen concentration in each portion and thereby compute the concentration of the different constituents In this case (Human Serum No 245A) the values are as follows free lipfanogens 6 4 complex 11 7 total lipfanogens 18 0 free antilipfanogen 4 3 total antilipfanogen 16 0 and precurser 32 4

Wakerlin In the second one you destroyed the antilipfanogen by heating?

Simms The second portion was heated immediately The third portion was incubated 3 days During those 3 days the precursor was converted by means of an enzyme into additional lipfanogens Then, in order to make the two comparable we heated the third portion at 90 C to kill the enzyme and the antilipfanogen The difference therefore is due to the additional lipfanogens produced by the precursor

Dock The control in ultrafiltrate would show no lipid at all in the cells?

Simms That is correct We first try to free the cultures entirely of fat granules by washing with serum ultrafiltrate The serum ultrafiltrate contains a stimulating agent which we call the A factor which maintains *the cells in the living state and supports* life but does not contain the lipfanogens In general we obtain cultures practically free from fat granules A control in ultrafiltrate remains free of fat

Ogden How long does the test run?

Simms Forty eight hours *Although the deposition is conspicuous* after 24 hours we have taken 48 hours as a standard 48 hours

TABLE V

No and Sex / Mean Age	Normals 5M 10F / 27	Diabetes 7M 13F / 46	Coronary Disease 15M 1.F / 53	Syndrome Nephrotic 7M 13F / 41
Fat Regulators				
L_F = Free LIPFANOGENS	7 6	9 3	9 4	8 2
ΔL = Bound "	21 8	16 2	13 9	11 0
L_T = Total	31 6	20 7	23 3	19 3
P = Precursor	11 3	7 5	13 2	14 8
A_F = − ΔL = Free ANTILIPFANOGEN	8 3	5 9	4 5	3 1
A_T = Total "	32 2	22 7	18 4	14 1
L_F/A_F = Free RATIO	0 92	1 57	2 09	2 62
L_T/A_T = Total "	0 97	1 16	1 30	1 57
Serum Lipoids				
Total Lipoids	846	1305	1137	1417
Phospho	189	282	270	286
Cholesterol				
Free	53	81	82	92
Ester	145	209	195	214
Total	198	290	277	306
Other Lipoids	458	733	590	825

Reprinted from article by Simms in *J Gerontol* 6 169 (1951)

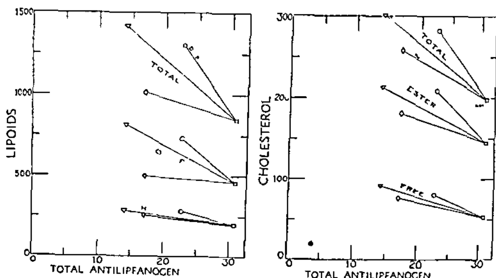


FIGURE 20 Relationship between total antilipfanogen and the lipid analyses

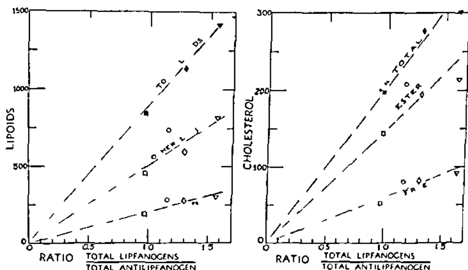


FIGURE 21 Relationship between the total L/A ratio and the lipid analyses Reprinted from article by Simms *J Gerontol* 6 161 (1951)

Shorr Does that chart include the precursor?

Simms The precursor is not included

A similar plot of free lipfanogens over free antilipfanogen which is probably more significant gives similar curves except that they are not straight In a given individual the ratio of lipfanogens to

TABLE V

	Normals	Diabetes	Coronary Disease	Syndr. of Nephrotic
No and Sex Mean Age	5M 10F 27	7M 13F 46	15M 12F 53	7M 13F 41
Fat Regulators				
L_F = Free LIPFANOGENS	7.6	9.3	9.4	8.2
$\triangle L$ = Bound "	21.8	16.2	13.9	11.0
L_T = Total	31.6	20.7	23.3	19.3
P = Precursor	11.3	7.5	13.2	14.8
A_F = $-\triangle L$				
= Free ANTILIPFANOGEN	8.3	5.9	4.5	3.1
A_T = Total "	32.2	22.7	15.4	14.1
L_F/A_F = Free RATIO	0.92	1.57	2.09	2.62
L_T/A_T = Total "	0.97	1.16	1.30	1.57
Serum Lipoids				
Total Lipoids	816	1305	1137	1417
Phospho "	189	282	270	266
Cholesterol				
Free	53	81	82	92
Ester	145	209	195	214
Total	198	290	277	306
Other Lipoids	458	733	590	825

Reprinted from article by *Simms in J Gerontol 6 160 (1951)*

antilipfanogen is remarkably constant Plotting one against the other, we get practically a straight-line curve which approximates a 1 1 ratio The ratio is a little lower for women than for men and it is lower in the dog and horse than in the human It is higher in chickens

There is presumably some regulating mechanism which controls the ratio of lipfanogens to antilipfanogen In a given individual the ratio remains constant while the values for the antilipfanogen and the lipfanogens vary from day to day At one time we thought the liver might be involved in the control of this ratio Subsequent observations on patients with liver disease did not support this

Table V is a summary of the values I have given Certain criticisms can be made The age groups of the normals are not comparable with those in diabetes coronary disease and nephrosis We intend to rectify this The preparation of the cultures is laborious and many experiments fail to give us satisfactory cultures So that it takes time to obtain the data

The first line under fat regulators gives the free lipfanogens which, as we see it are the materials directly involved in the fat deposition The average for our normals is 7 6 diabetics 9 3 coronary disease 9 4 and nephrotic syndrome 8 2 The individual values *of course vary considerably, and in themselves are not significant* The significance comes only when we have enough values to obtain averages But these differences although they may seem small represent a 20 to 25 percent increase in free lipfanogens above the normal value Over a period of time this may be sufficient to cause a deposition which would not occur otherwise The table also gives values for the bound lipfanogens total lipfanogens precursor and antilipfanogen (free and total) At the present time we have not found any significant relationship between the precursor and these diseases

Katz Dr Simms do you consider the lipfanogens to be enzymes?

Simms No the lipfanogens are the fatty materials that are converted into visible fat The *free* lipfanogens represent the fat depositing potential

The left half of this chart (Figure 22) is the same as the first chart representing the free lipfanogens the complex the free antilipfanogen, and the precursors

The right half of this chart represents the conditions in the diseases we are studying — coronary disease diabetes and nephrotic

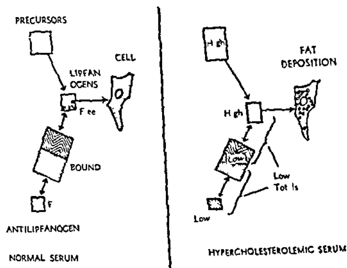


FIGURE 22 The left side of this diagram is the same as Figure 15 The right side shows the changes in hypercholesteremic serum

syndrome The total lipfanogens are low the total antilipfanogen is low the free antilipfanogen is also low *but the free lipfanogens are high* Consequently there is more fat deposition (as indicated by the fat granules in the cell) These comments refer only to the cells in our tissue cultures

Stamler Is the free lipfanogen independent of the neutral fat which is elevated in this hypercholesteremic serum?

Simms The free lipfanogens (as well as the total lipfanogens) represent a special group of lipids Whether they represent any considerable portion of the neutral fat that is present in the plasma I cannot say My guess would be that their concentration is independent of the concentration of neutral fat

Gofman Have the lipfanogens been isolated in any way by themselves?

Simms No We have not attempted to isolate the active lipfanogens from the inactive fats We have worked on the antilipfanogen more than on the lipfanogens

Barr Do you know what Cohn fraction the lipfanogens come from?

Simms Lipfanogens are present over the whole range of precipitation Every fraction has some lipfanogens They are somewhat

more concentrated in the globulin fractions than in the albumin It is one of our problems to get rid of them in fractionating the antilipfanogen

Ogden When you heat the serum for the destruction of anti lipfanogen, do the lipfanogens come down with the protein or do they stay in solution?

Simms The lipfanogens come down when there is protein pre cipitation However, we try to prevent precipitation by diluting the serum with distilled water before heating The necessary salts are introduced afterwards

Ogden How do you free them from the protein?

Simms In our serum tests we do not free them Any serum that gives a precipitate is discarded The only way we have freed lip fanogens from protein is by extracting with ether and as I men tioned earlier, about half the lipfanogens are ether soluble We have not attempted to go beyond that The next chart (Figure 23)

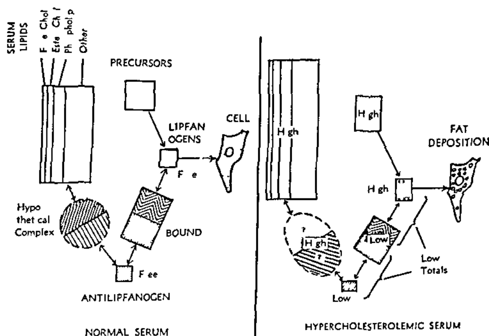


Figure 23 This is identical with Figure 22 except that the serum lipids have been added and also the hypothetical complex between the (inactive) serum lipids and the antilipfanogen

brings up the question of the serum lipids These are indicated by the rectangles in the upper left corners Whereas in Figure 15 there was a proportionality between size of the squares and the amount of the agents in this illustration no proportionality exists Unfortunately I have no idea of the weight concentration of our agents compared with the serum lipids The rectangles are divided into areas corresponding to the free and ester cholesterol phospholipids and other lipids As we know in hypercholesteremia these substances are all higher

To come to the question as to the upset in the balance of the lipfanogens and antilipfanogen in hypercholesteremia hypothetically there may be a complex between the free antilipfanogen and one or more of these analyzable serum lipids If that is so and the circle in Figure 23 represents the complex then in hypercholesteremia there would be a greater tendency to combine with the antilipfanogen (as represented by the oval) thereby reducing the concentration of antilipfanogen which otherwise would be available for combining with the lipfanogens In other words if this theory is correct there is competition for combining with the antilipfanogen between the lipfanogens on the one hand and other serum lipid material on the other hand So that when the lipid material binds more of the antilipfanogen that amount of the antilipfanogen is then not available to bind the lipfanogens thus resulting in a high free lipfanogen I cannot say positively that this is true but at least it fits in with our observations

Goldblatt In other words you make this determination first and find the level of the lipfanogens then you incubate and you show there is an increase

Simms Yes

Goldblatt And you call that the effect of the *precursor*?

Simms Yes The increase in activity after incubation represents lipfanogens produced from the precursors

However we dont know the source of the lipfanogens although they are present in serum and practically all the tissues We dont know the source of antilipfanogen It is found in blood plasma but in negligible amounts in the tissues Obviously it is produced somewhere but we dont know where

We dont know what produces the precursors or whether the precursors have any relationship with the hypothetical complex

more concentrated in the globulin fractions than in the albumin It is one of our problems to get rid of them in fractionating the antilipfanogen

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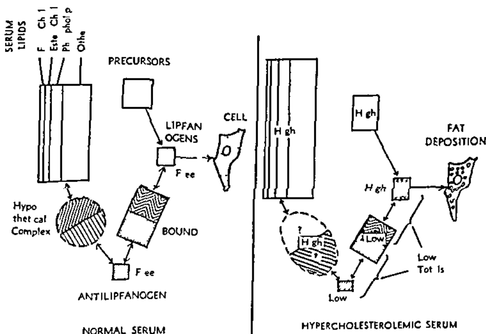


Figure 23 This is identical with Figure 22 except that the serum lipids have been added and also the hypothetical complex between the (inactive) serum lipids and the antilipfanogen

Katz Has it ever been ultracentrifuged to determine its flotation number assuming of course that it is protein or protein bound?

Simms No However Dr Gofman Dr Barr and I are about to start on a cooperative project in which we will make tests on the same samples of serum Furthermore some of their purified fractions will be tested in our laboratory and we are hoping that we will be able to dovetail our information and find the relationship between the various agents with which we are working

There is one thing that I have forgotten to mention so far — giving thyroid by mouth raise the antilipfanogen — apparently a liberation of antilipfanogen rather than a production

Dock I should like to know how many people here believe that in the development of atherosclerosis the lipid is first visible in cells and how many think it is first visible in intercellular substance Your group has been looking into this Dr Stamler What do you think?

Stamler This is one of the most difficult problems facing investigators in the atherosclerosis field Both cholesterol fed and stilbe terol treated cockerels exhibit diffuse lipid infiltration of the intima and media of the great vessels early such lipid is found both extra and intracellularly The relationship of this finding to the subsequent development of atheroma and atherosclerosis in these chicks is not clear This diffuse lipid infiltration is not necessarily associated with plaque formation or a fibroblastic proliferation reaction There may be no thickening of the vessel wall or impingement of the vascular lumen Many vessels exhibit this finding without associated plaque formation When plaques are present they are focal whereas the lipid infiltration is diffuse Is the latter pre atheroma? If the microscopic intimal foam cell plaque (so called "pure" atheroma) be accepted as the initial morphologic stage of atherosclerosis *per se* then the lipid is invariably intracellular With evolution of the plaque foam cell degeneration apparently occurs and the lipid becomes extracellular Of course this pathologic sequence is a presumed one deduced from autopsy material Its validity has not in our opinion been finally established We would be interested to know whether studies such as Gofman has accomplished with labelled cholesterol reveal radioactive lipid both in foam cell (lipohage) plaques and in the diffusely infiltrated vessel free of plaques

Goldblatt I was going to ask Dr Stamler to define or describe a plaque

Gofman Dr Simms did you say that ether extracts retain any lipfanogenic activity?

Simms Yes we can take it up again in aqueous solution The ether extract contains about 50 percent of the lipfanogenic activity Incidentally the equilibrium constant for combining with the antilipfanogen is essentially the same for the ether soluble portion as for the ether insoluble

Goldblatt If you keep the culture going in serum ultrafiltrate for a week or ten days, do you see so called fat phanerosis that is fat becoming visible in the tissue and presumably not from the medium?

Simms There is a slight initial fat phanerosis of the original tissue *due to lipfanogens in the tissue — but the new cells remain* free from fat granules as long as we keep them in serum ultrafiltrate (up to 100 days)

Katz Dr Simms have you used a culture medium with polyvinyl alcohol added to it?

Simms We tried it but it apparently did not diffuse through the medium to the cells

Katz Can the amount of fat appearing in the cells be accounted for quantitatively by the lipfanogens in the serum?

Simms Yes We have made quantitative curves in which the fat deposited has been found to be proportional to the free lipfanogens in the medium

Wakerlin Have you extracted other tissues for antilipfanogen? I am thinking particularly of the kidney

Simms Yes we have extracted practically every tissue trying to find the source of the antilipfanogen There were traces in the spleen and in the brain Also by taking a pancreas extract and fractionating it we *obtained slightly more activity But in no case* was there enough activity to indicate that we were dealing with the source of the antilipfanogen

Katz Could you prepare enough lipfanogen so that it could be ultracentrifuged?

Simms It is easy to prepare plenty of crude lipfanogen However the concentration of active lipfanogens in this crude material is unknown

dyes (ordinarily taken up by macrophages) intravenously, after atheromata had been established They observed no uptake of the dyes by the foam cells of the atheroma Even if these were macro phages that had migrated their experiment might have failed be cause the macrophages didn t have the opportunity to become directly exposed to the dye

We approached the problem by labelling the macrophages of liver (Kupfer cells) spleen and marrow, using a thorium oxycitrate colloid The thorium had the isotope ionium added to increase the alpha radioactivity With this technique one could actually demon strate alpha particles emerging from the macrophages and not from parenchymal liver cells Cholesterol was then fed for 90 to 100 days on the assumption that if macrophages took up cholesterol and migrated to the aortic wall we should find alpha emitting cells in the aortic plaques The experimental result was a completely nega tive one No evidence was obtained favoring the hypothesis that macrophages migrate from the Kupfer cells of the liver or from the reticuloendothelium of the spleen or bone marrow to become the foam cells of atheroma The labelled macrophages remained functional throughout maintaining their phagocytic capacity to accumulate India ink injected into the bloodstream Further lipid had also accumulated in these cells This experiment does not sup port the hypothesis of cellular migration in atheroma formation It increases our belief that the blood itself may be the source of the lipids in atheroma formation

Stamler You say that these liver cells do contain labelled cholesterol after the animals have been given a tracer dose?

Gofman Yes and the foam cells of atheroma show no bursts of alpha particles indicating any migration

Stamler Dr Duff I think reached a similar conclusion based on some work with thorium

Dock If cells were merely migrating from the liver there would be no reason to pick out the aorta rather than the vein to settle in After all although the pressure is lower in the pulmonary artery it is notoriously free from such lesions Leary would have to explain why these macrophages ignored the whole lining of the pulmonary artery capillaries and veins and then why they invaded the systemic arteries

Gofman Leary in 1950 published a paper showing evidence that he takes for migration namely showing a macrophage actually wending its way through the endothelium of the aorta

Stamler In chicks the earliest true lesion i e , a change producing thickening *of the vessel wall is a focal microscopic foam cell* intimal cushion made up of lipophages, swollen with lipids including anistropic lipids This cushion intrudes into the lumen narrowing *it even if only microscopically* A more developed gross plaque which presumably evolves from this initial plaque exhibits intimal fibrotic thickening plus atheromatous abscess formation deposition of cholesterol crystals, and compression of the media In the chick intimal thickening is easy to determine because the normal chick intima is one layer thick It becomes several layers thick due to connective tissue proliferation with lipids interspersed amidst the fibrous elements This process evolves to the stage of calcification and even cartilage and bone production — everything that you see in the human except ulceration

What we are unsure about is the pathogenetic relationship between such focal plaques and diffuse lipid infiltration of the arterial wall completely unassociated with foam cells fibrotic reaction calcification and thickening

Goldblatt And you see no sign of this process in the media?

Stamler In some cases of diffuse fatty infiltration fat gets into the media too but without producing any proliferative changes or lipophage accumulation But when plaque formation occurs it seems to occur in the intima Only secondarily is the media involved

Does your work Dr Gofman with labelled lipoproteins enable you to distinguish in a time and pathogenetic sense between diffuse lipid infiltration and plaque formation?

Gofman The data are not good enough to say much on that score Labelled cholesterol shows what you would count as rough turnover time This is in the order of 10 to 20 days in the aorta of rabbits not receiving any cholesterol feeding whereas for plaque cholesterol as a rough estimate the turnover time was between 20 to 40 days from Dr Biggs data

Dock Do you have any radioautographs to show the initial point of deposition of labelled cholesterol as to whether it is extracellular or intracellular when it first gets into the aorta?

Gofman No we do not Simonton and I tried to test the hypothesis originally put forth by Leary that lipids enter atheromatous areas via macrophages that have migrated from such organs as the liver Kuntz and Sulkin also tried to test this by giving certain

resultant proliferation of fibroblasts in that site and later fatty deposition as a consequence of the degenerative changes occurring in the inflammatory tissue I held the view that deposition of lipid material was the result of a primary infiltration of the intima with lipids from the blood

In connection with a statement made by Dr Dock I would like to ask why a vein relatively seldom becomes the seat of atherosclerosis I should like to hear Dr Dock and others speak about why the veins may not show any atherosclerosis even when it is pronounced in the aorta and large arteries

Dock You have to have a certain level of what we might call percolation pressure before atherosclerosis will develop anywhere In people who have varicose veins and insufficient valves in the veins of the legs atherosclerosis does develop below the knees It is my impression that pressures over 60 mm Hg result in atherosclerosis when the blood lipid pattern is also abnormal

Katz In cholesterol fed rabbits or chicks with marked chronic hypercholesteremia lesions occur in the veins and pulmonary arteries despite the low "percolation" pressure However with smaller percentages of dietary cholesterol (e g ¼ percent) which induce only minimal hypercholesteremia and organ lipidosis lesions are confined to the systemic arteries of chicks The pulmonary and venous trees remain free of lesions

Simms I might point out that different fibroblasts in the body have different propensities to form fat granules In a single spot in the aorta for example if we start at the intima and work outward we find an increasing tendency to form fat granules in the fibroblasts Similarly taking fibroblasts from different arteries as for example the carotid fibroblasts compared with the aorta fibroblasts we find a difference in the tendency to form fat granules We must recognize that there are tissue differences which may be responsible for the localization of the plaques

Grimson It occurs to me there is some additional material that can be considered Grafts of vein or artery are being used surgically relatively frequently in dogs and in man In those specimens from sacrificed dogs that I have seen at surgical meetings there occur not infrequently white or yellow deposits or plaques Histological studies presumably indicate that only a small part of the original artery or vein remains viable or in other words that the graft is a splint and that fibroblastic tissues develop over and about it and

Dock You cannot tell in a fixed section whether it is going in or out

Shorr Dr Simms, to what extent has the purification of the antilipfanogen gone? Do you think that it is a large portion of the albumin fraction or that it is one small specific component of it?

Simms Apparently, it is a special component The yield of active material at the present time is very small Unfortunately, the loss of activity of purified material is so rapid we cannot determine the activity with accuracy

Ogden Dr Simms, you found that in the cholesteremic states the free lipfanogens were increased Do you know whether that applies both to the ether soluble and ether insoluble fractions?

Simms I am not able to say because we cannot make extracts of the free lipfanogens in the absence of the bound

Ogden When you speak of the free it is both fractions?

Simms Both ether soluble and ether insoluble Of course if we started extracting we would break up the complex and would not be dealing with free lipfanogens The total lipfanogens are low in hypercholesterolemia but the free are high We dont know whether the increase in free lipfanogens represents an increase in the ether soluble type or the ether insoluble type or both

Ogden Your extract only gives you half of the total?

Simms Yes about half the total lipfanogens are present in the ether extract

Gofman I should like to ask one question and then make one suggestion if I might The question is I think those of us who are suspicious that lipids are involved in atherosclerosis have a certain number of opponents in that some very good pathologists have stated that they believe lipids are not part of the primary development of atherosclerosis I know Rinehart in California feels that the primary process in alterations of this type is fibrosis and that the lipid deposition is wholly secondary I personally do not subscribe to this I wonder whether anyone here feels that he could state definitively the sequence of events in the development of atheroma

Goldblatt In the Department of Pathology at Western Reserve University for twenty four years Dr Karsner and I taught opposite views about the pathogenesis of arteriosclerosis Dr Karsner believed that the primary process was inflammation in the intima with

dogs in order thereby to destroy the vasa vasorum on the assumption that the blood supply to the arterial wall is of significance in the causation or prevention of atherosclerosis He then gave some animals cholesterol others cholesterol plus thiouracil Within a couple of months atheroma like lesions developed in the intima subjacent to the site of adventitial cauterization No other part of the aorta was involved

Goldblatt This lesion was in the media?

Katz No in the intima overlying it I am assuming Dr Grimson is talking about atherosclerosis and not fibrosis It is possible that the transplants having a poor vasa vasorum to the walls may be more vulnerable if hypercholesteremia is induced in the dog

Grimson Since I have not seen sections or done the work Dr Katz s question cannot be answered I pointed out that arterial or vein transplants into the area are a means of study that might be worthy of detailed examination These yellowish plaques do appear on the colored lantern slides that have been presented at several meetings The nature of these plaques and their location may have some bearing on the problem of arteriosclerosis

Stamler We have seen dogs with such changes but they are not atheromatous lesions microscopically

Dock They may be blood pigments

Stamler Is it generally accepted that syphilitic lesions in the media are sites of predilection in human beings for secondary atherosclerosis?

Dock Yes Radiologists diagnose syphilis of the aorta by seeing chalk in the ascending aorta which they almost never see in people with dilated aortas without syphilis In the same way at autopsy we separate the two by the presence of syphilitic medial aortitis Dr Snapper(1) tells me that in Peking such lesions do not accompany syphilitic medial aortitis and Dr Woo the pathologist at Peking states that syphilitic medial aortitis occurs in elderly Chinese gentlemen in whom there were no atheroma anywhere else including coronary arteries There was simply syphilitic medial aortitis with thickened intima but without any atheroma in this thickened intima

Wilens I can confirm this observation from my own experience In Central America where syphilis of the aorta is fairly common atheromatous lesions are seldom superimposed on the luetic process

form much of the new channel Some of the original elastic tissue may remain viable a year or more These grafts afford a situation *in which a type of arteriosclerosis* can occur and can be studied and in which the artery or vein has been separated from its intrinsic circulation and is in direct contact with the blood stream

Goldblatt What did you *transplant to where?*

Grimson Many surgeons are removing veins or arteries from dogs, storing or refrigerating them and then using them as grafts after various periods of time Human arteries removed during autopsy are put into patients Grafting is a successful procedure but these plaques do occasionally occur in transplants of either vein or artery

Goldblatt That interests me very much because several years ago Dr Heringman and I put to the test the effect of increased blood pressure on the development of phlebosclerosis by trans planting a portion of the *jugular vein about an inch and a half* long into the femoral artery of normal dogs We intended later to do the same in some hypertensive animals but never did I can report the results in only five normal dogs In three of these after one year and in the other two after about six months we saw no signs of atherosclerosis in the transplanted veins In one animal in which we did find a plaque of intimal thickening it was due to a healed mural thrombus There was no sign of atherosclerosis in this plaque

Dock But there was none anywhere else in the dog

Goldblatt That is correct

Wilens I should like to disagree with Dr Dock about the occur rence of atherosclerotic lesions in varicose veins I have examined a large number of such vessels that have been removed surgically They frequently show mild degrees of intimal fibrosis but almost never have deposits of lipid in fact they seldom become calcified

Grimson I will have to challenge that in that I have once seen bilateral calcification of the small saphenous veins

Wilens In my experience extensive calcification of varicose veins occurs only when the vessel has been thrombosed in which case the thrombus rather than *the vessel will becomes calcified*

Katz I should like to comment on some experiments that were done in my department by Dr Schlichter which may clarify what Dr Grimson *mentioned* Dr Schlichter cauterized the adventitia of

sclerosis involving all the major branches of his coronary arteries and leading to myocardial infarction and death Xanthoma appeared in the skin before the child was a year old and his blood cholesterol level was 540 Again here was an example of a high blood cholesterol level associated with deposition in the connective tissue and in the skin and with atherosclerosis

Katz In chicks fed 1/4 percent cholesterol mash beginning with the first day of life lesions developed by 12 15 weeks although mean plasma cholesterol level was only 140 mg percent (controls 90)

Goldblatt In other words it is a hypercholesteremia for chickens

Katz Yes to a very mild degree without associated organ cholesterosis

Goldblatt Why do you say "mild degree" when it is double the normal?

Katz Because that is the sort of range that one is talking about when one speaks of a xanthomatous tendency in people with coronary atherosclerosis

Goldblatt Oh yes but then I do not think that it is quite fair to make that comparison

Katz Without necessarily insisting that the source of the slight hypercholesteremia is identical (exogenous vs endogenous) we feel the experiments are highly germane to the problem in man They demonstrate the role of altered cholesterol metabolism in the pathogenesis of atherosclerosis

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2 HUEPER W C Experimental studies in cardiovascular pathology thesaurosis and atheromatosis produced in dogs by repeated intravenous injection of sodium cellulose glycollate *Am J Path* 21 *1021 (1945)*

3 HUEPER W C Atheromatosis in dogs following repeated intravenous injections of hydroxy ethyl cellulose *Arch Path* 41 *139 (1946)*

In New York, on the other hand severe atherosclerosis almost invariably accompanies the luetic lesion

Dock Atherosclerosis in the coronaries is relatively rare

Wilens Yes, it is rare

Lansing Do you see intimal fibrosis?

Wilens Yes

Lansing Is it not possible that intimal fibrosis is the primary event, or earlier lesion with the cholesterol accumulation a secondary event secondary but deadly?

Dock The question remains as to whether the appearance of cholesterol in the thickened intima is a degenerative change or a deposition analogous with the deposition of polyvinyl alcohol in a dog where the same sort of lesions appear under conditions obviously not the result of degeneration When Dr Hueper(23) gives higher alcohol polymers to dogs he finds lesions which resemble atherosclerosis except that whereas Dr Kendall's dogs have cholesterol the lesions here are filled with synthetic macromolecules These macromolecules must have entered the plaques from the blood plasma They could not possibly have come by either synthesis or degeneration However the question remains whether atheroma formation is analogous to Dr Hueper's deposition or whether degeneration occurs in tissue which undergoes a biotropism or some other sort of change that leads to deposition of cholesterol

Katz Chickens, with a life expectancy of up to 20 years when fed a 2 percent cholesterol diet from hatching on develop lesions as early as the fifth week of life By the tenth week severe atherosclerosis is present in both the thoracic aorta and the coronary arteries It would appear therefore that cholesterol will produce lesions of the atherosclerotic variety in vessels subjected minimally if at all to so called wear and tear of age or to previous vascular damage or to ground substance senescence etc While it is possible therefore that syphilis infection and other injuries or aging of the intima may create sites for secondary deposition of atherogenic lipid one cannot deny the possibility that atherogenesis may proceed in previously normal vessels given the prerequisite atherogenic alteration *in lipid metabolism*

Dock We autopsied a 3½ year old child at Kings County Hospital about three months ago who had died of coronary athero

I have always felt that in dealing with humoral mechanisms we should whenever possible use pure substances We have therefore been working on the isolation of pure renin Table VI shows that more than five years ago Dr Yale Katz and I had already obtained renin with a titre of 130 dog units per mg nitrogen and later Marshall and Wakerlin also produced a hog renin which had about 125 dog units per mg of nitrogen Like ourselves they used relatively small quantities of kidney and did not use renin of this purity for the production of antirenin in animals

In planning to produce antirenin to hog renin in man Dr Haas Dr Lamfrom and I thought that to avoid untoward reactions it would be necessary to use highly purified hog renin Because of the difficulty in obtaining dogs for the tests in Los Angeles and because we wanted a good yield as well as purification it has taken about four years to do what might have been accomplished in one year Since there is no chemical test for renin the biological one is still the only reliable guide As you see from Table VI, we now have a product with 5000 units per mg of nitrogen which is about forty times as pure as the purest renin previously mentioned in the literature

We have subjected the material to various tests for absolute purity and although we still do not have it in crystalline form we feel that we have almost exhausted the known methods for the absolute purification of this protein

In our earliest tests on human beings we found that renin which titrated at about 180 dog units per mg N (Table VII (step 6) could be safely administered With this type of renin antirenin was readily produced in the dog We realized *a priori* that hog renin injected into man might not induce the development of antirenin and even if it did that this antirenin might not inactivate human renin *in vitro* and would therefore not lower the blood pressure of hypertensive individuals To our agreeable surprise antirenin to hog renin did develop as a result of the subcutaneous injection of purified hog renin into hypertensive human beings Table VIII shows that the highest titres of antirenin to hog renin reached in man were even higher than the highest titres (20 units per cc) we had ever observed in dogs Unfortunately however this antirenin was not effective against human renin *in vitro* and produced no effect on the blood pressure that could not be attributed to the injection of a foreign protein

EXPERIMENTAL HYPERTENSION

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I HAVE BEEN asked to open this discussion with a progress report on the work that we have been doing during the past few years One of our main problems has been the mechanism of formation and release of renin from the kidney into the circulation We have been trying to determine whether it exists in the kidney as pre formed soluble renin or whether a precursor is changed to renin at the time of its release into the blood stream by the removal of an inhibitor, or by the action of some other enzyme or catalyst I am able to state only that we *believe* that a renin precursor, which we have named crypto renin is transformed into the *soluble active form in which it enters the circulation but the exact* nature of this transformation has not yet been elucidated

I have been accused of emphasizing too much the humoral mechanism of experimental renal hypertension and perhaps rightly so but having performed the first experiment which indicated that as a result of constriction of the main renal arteries a chemical substance enters the circulation which brings about the elevation of the blood pressure I am naturally greatly interested in the humoral mechanism of experimental renal and possibly of human hypertension

TABLE VI

Comparison With Previous Methods

AUTHORS	YEAR	Kg KIDNEYS	UNITS RENIN / mg Nitrogen
Helmer and Page	1939	1	3
Collings Remington et al	1940	3	6
Schales	1942	1	17
Katz and Goldblatt	1943	4	130
Marshall and Wakerlin	1949	10	125
Haas Lamfrom Goldblatt	1951	70 140	5000

As far as I am concerned I do not see a way out of this difficulty unless it would be possible to make human renin antigenic and antirenin to human renin would develop as a consequence of the injection of such altered human renin Except for academic reasons the production in man of antirenin to anthropoid ape renin would not be a practical contribution It would probably be difficult even to obtain enough human kidney for practical purposes judging from the amount of hog renin that is necessary for the treatment of experimental renal hypertension in the dog

You may well ask "Does not this prove that human hypertension is not of renal origin and even if it is of renal origin that a humoral mechanism similar to that of the experimental animal does not obtain?" The answer to both questions is "No" because the hog antirenin developed in man is not antirenin for human renin even in vitro We are therefore trying to produce a large quantity of antirenin to human renin in an animal and we will give this antirenin against human renin to a hypertensive human being to see whether it will have a brief effect on the blood pressure This might help to clarify the matter of the renal humoral mechanism in human essential hypertension

Helmer If I may interrupt we thought of doing the same experiment However we were worried for fear the patients might develop a Masugi type nephritis That might not happen with your purified renin

Goldblatt I thought of using the malignant type of hypertension in the hope that at least a brief improvement might occur but such an experiment might be hazardous

Dexter Have you thought of trying to make human renin antigenic to human beings? There are definite leads for making human proteins antigenic to the human species

Goldblatt Yes I believe I mentioned that possibility Again except for academic reasons this would not be fruitful because judging from the amount of *hog renin* we have been obliged to use it would be impossible to obtain enough human kidneys for this to be of any practical value

Dexter During the war I collected all the kidneys in Boston from almost every hospital We put them in a deep freeze and kept them there Over the course of time we would get big batches of kidneys

TABLE VII

Procedure for Isolation of Renin

		$\frac{\text{UNITS RENIN}}{\text{mg Nitrogen}}$
Extract of kidney tissue (water and benzene at 23° C)		0.09
Autolysis Benzene pH 8.5 38° C		0.36
Ethanol fractionation at pH 2.1		5.8
Sedimentation of renin tungstate		
$(NH_4)_2SO_4$ – Fractionation pH	4.3	23
Acetone	" 4.8	60
$(NH_4)_2SO_4$*	5.5	180
	7.5	300
	" 3.9 25° C	700
"	" 2.5	1200
Acetone	3.5	5000

* This was the renin used for injection into human beings

TABLE VIII

Antirenin in Hypertensive Patients

PATIENT	TREATMENT weeks	RENIN INJECTED units	$\frac{\text{units}}{\text{cc serum}}$ ANTIRENIN
Mrs B T	3.5	28 000	0.8
Mr H W	5	20 000	0.4
Mrs L E S	6	48 000	0
Mrs B C	7	56 000	11
Mr V M	8	64 000	1.0
Mrs S K	20	112 000	16
Dr I Y B	20	132 000	37
Mr H B	33	142 000	2.0

took thirty seven dog units of hog renin added one unit of human renin and to this mixture added one cc of human serum containing 37 units of antirenin to hog renin There was a response of one unit which it is fair to interpret meant that the 37 units of hog renin were inactivated and that the response of one unit was the effect of the one unit of human renin It was a very convincing experiment Then we did the reverse just used one unit of human renin and added 37 units of antirenin to hog renin There was no effect whatsoever on the human renin Despite this negative result I still cling to the possibility that the renal humoral mechanism may be involved in human essential hypertension We are also attempting to detect the presence of renin and hypertensin in the systemic blood of patients with essential hypertension One of my former associates Dr Joseph R Kahn at the Crile Veterans Hospital in Cleveland in collaboration with Dr Skeggs using the dialysis in *vivo* technique is also working on this problem By this method he and his collaborators have succeeded in demonstrating hypertensin in the circulating systemic blood of animals that had had hypertension for not longer than three months Before that by the direct method we had obtained the same result

Helmer When you consider how little angiotonin it takes to raise the blood pressure of normotensive individuals to hypertensive levels the difficulties of detecting it in the blood stream can be appreciated Five of our units will do this With the present degree of purity 25 micrograms is equivalent to a unit therefore 125 micrograms would be present in the circulation at any *one* time Actually much less might be present since the angiotonin being used is far from pure We are looking for too much in too little The dialysis methods of Dr Kahn may be a means of concentrating pressor material from a large quantity of blood

Ogden What do you think about the experiments of Gregory s in which he injected enough angiotonin *to* produce blood pressure elevation and showed that *he* could detect it by testing the blood? Comparable assays failed to show angiotonin in his patients with essential hypertension

Helmer I think I may be able to explain his results I supplied Gregory with the angiotonin he used in his experiments It was the same preparation Dr Shorr tested for VEM activity(1) He reported a 4 plus VEM activity

Gregory tested the vasoconstrictor effect of angiotonin by perfusion of whole pithed frogs He found that the plasma from normal

and then we would process them for human renin, having kept them packed in CO_2 ice thoroughly frozen

Goldblatt Dr Kahn, at the Veterans Hospital in Cleveland is collecting kidneys in a deep freeze for us I expect both Dr Wakerlin and Dr Helmer, about whose work we learned after we were all through with our antirenin work on the human being to take over from here

Dock None of your animals developed nephritis from these injections?

Goldblatt No

Dock You have not used the serum from one animal to another?

Goldblatt No We developed antirenin in animals before and after constriction of the renal arteries The effect of injecting a large quantity of antirenin into a hypertensive dog should be tried It would require a large amount I guess

Helmer According to some Scandinavian workers Masugi nephritis can be prevented by administration of antihistamines This might offer some hope

Dexter Perhaps antirenin production would also be inhibited

Have you given your purified hog renin to dogs with hypertension and reaffirmed your previous results?

Goldblatt Yes renin as purified as the material we used in hypertensive human beings is effective In Table VIII is a summary of some of the results obtained in our best patients We had fourteen but only eight are listed because for one reason or another enough injections were not given to the other six The highest titer developed was 37 units per cc in one patient This is larger than any amount that we had ever produced in a dog yet even in that person there was no significant effect on the blood pressure after twenty weeks of injections That applied to all the others no matter what the titer of antirenin was It had no effect on the blood pressure

Dexter You are referring I assume to an antirenin titer to the injected hog renin and not to the patient's own renin

Goldblatt This antirenin was effective against hog and other animal renins We tried hog sheep and ox renin It neutralized these other renins in various titers but not human renin We even

Helmer The only species we found that produced antirenin against its own renin when injected with hog kidney extracts was the dog In addition to the dog we tried cats rabbits and human subject The plasma from our human subjects treated with hog renin would even "neutralize" rat renin, although human renin is practically vasoinactive when injected into rats The human plasma would neutralize" the renin from the kidneys of hogs dogs rabbits cats sheep horses and rats but not that from human kidneys The rat behaves exactly like a human

Wakerlin You made a comment on the question of the site of formation of renin A student of mine J Marshall(3) did some work several years ago which indicated that renin might be formed by the cells of the juxta glomerular apparatus There was an excellent correlation between the number of granules in the J G A having histochemical properties similar to those of renin and the renin content of dog kidneys

Goldblatt I am surprised in a way because the dog is one of the animals that shows few of these granular cells

Wakerlin A more critical experiment than this is one which has recently been done by Taquini Fasciolo and Fernandez Luna in South America and reported to me by Dr Braun Menendez when he was here In this experiment very thin segments of cortex from the dog kidney were shaved off the surface so that *there were no glomeruli and no J G A* and renin was definitely *present in the* renal tubule cells The granules that Marshall worked with must have been something other than renin but apparently well correlated with the renin content

We have not been satisfied with the purity of our hog renin of 120 dog units per mg N but Dr Osgood our biochemist has concerned herself with renin production rather than renin purification during the past two years The best preparation we have had so far is 140 dog units per mg N

Of importance in relation to the antihypertensive effect of antirenin is the absence of medullary protein from the preparation Certainly with crude hog renin prepared from whole kidney there is an important interfering effect from medullary protein This carries over to some degree in the purification that we have achieved so far which is not nearly so *great as yours* This might be of significance in regard to Dr Helmer's experiments on the rat since I understand he used whole hog kidney as a source of renin

and hypertensive *patients had the same amount of constrictor* activity. However, the plasma of patients whose blood pressure had been raised to hypertensive levels by injection of angiotonin had a much greater amount of vasoconstrictor substance. These experiments have been interpreted as indicating that angiotonin is not an etiological agent in essential hypertension in man. It is possible that Gregory was testing for the vasoconstrictor effect of the VEM impurity in angiotonin rather than for angiotonin. Therefore his work does not necessarily disprove the renin angiotonin system as a factor in the production of essential hypertension.

I hope that the angiotonin that has been purified by means of chromatography(2) may be free of VEM. If Gregory had had the opportunity to use a renin free of VEM his results might have been different.

Zweifach There is no evidence that VEM produces a vasoconstrictor effect *in perfused preparations. On the contrary* the only vascular effects of VEM thus far obtained have been on the responsiveness of the terminal arterioles and precapillaries to topical epinephrine in the mesentery of the intact animal.

Goldblatt I did not personally accept Gregory's results as a telling blow against the renal humoral mechanism of hypertension.

Wakerlin Have you had a chance to reexplore the pharmacology of the purified renin as Dr Marmorston has been using it for repetition of studies with Dr Addis?

Goldblatt Yes. I can tell you that 4 units of renin which titrated at 800 units per mg of N injected intraperitoneally into rats had a proteinuria effect as effective as 4 units of the crudest renin that has been used for this purpose.

Helmer Didn't angiotonin do that too?

Goldblatt The effect is more fleeting.

Helmer Just to add one more species to those on your slide, Dr Goldblatt, we gave the same hog renin to hypertensive rats that we gave to hypertensive *patients. The titer of antirenin in the* rat plasma after several months was 800 Goldblatt units per 100 cc. There was a high titer against hog, cat, rabbit and dog renin but practically none against rat renin. There was no fall in blood pressure.

Goldblatt It did not neutralize rat renin?

Fremont Smith Do you have to keep up the injections during the time you apply the clamp and during the time when you would anticipate the hypertension?

Wakerlin Usually but not necessarily In other words when the antirenin titer drops off – and it does, several weeks after you stop the injections – then the blood pressure goes up You can not only keep the blood pressure from going up during the period of constriction of the renal arteries and for a short period afterwards but by continuing the injections and maintaining the antirenin titer the blood pressure can be kept from going up indefinitely – I mean for months or years by *continued* periodic application of the antigen

Fremont Smith Is there enough reaction to the injection in the terms of protein reaction or febrile reaction to cause the antihypertensive effect?

Wakerlin Such a reaction cannot possibly account for our results We have many negative experiments using crude hog *renin from* whole kidney and other tissue extracts With crude renin from whole kidney we had good antirenin titers but no antihypertensive effect A nonspecific foreign protein effect is ruled out by hundreds of experiments actually We have also checked the body tempera*tures of the* dogs Except for a rare local infection these animals show *no reaction* local or general to the injections This is especially true of the more purified renin preparations where the quantity of injection is small

Stamler Does the pressure go up when you stop injections in these dogs with clamps?

Wakerlin As the antirenin titer drops down That takes six eight or ten weeks

Fremont Smith For six or eight weeks the blood pressure remains down and then gradually goes up again?

Wakerlin That is right For reasons which are not clear some years ago we had dogs that *remained* normotensive for about three years after we stopped injections That material was crude hog renin contaminated with medullary protein For the last four years our technique has been so well *standardized that* every control dog that we constrict becomes hypertensive Ten years ago when we first showed that one could *protect against* hypertension our degree of constriction of the renal arteries was not as well standardized as it is now Our constriction was somewhat less severe than now and

Helmer But I was going by the antirenin titer

Wakerlin I have seen antirenin titers of 40 A U per cc of serum without any hypertensive effect when the crude hog renin (1 dog unit per mg N) used in treatment was prepared from whole kidney On the basis that antirenin to hog renin does not neutralize rat renin, which our group showed years ago, one would not expect a decrease in blood pressure in hypertensive rats but I think that the experiment would be more valid if renin prepared from cortex rather than whole kidney was used

Helmer The same renin reduced the pressure of three dogs to normal and produced a good titer when injected into rats but did not lower the rat s pressure

Wakerlin Your purification obviously removed sufficient of the interfering medullary proteins At any rate, I think before drawing negative conclusions it would be better to use cortex rather than the whole kidney as the source of renin

In regard to vascular lesions and experimental malignant hyper tension our research group (particularly Mr R O Burns) has reported that one can protect against the hypertension of malignant hypertension with antirenin We are fairly certain however that the protection against the vascular lesions is not due to antirenin but is due to something in the nonrenin fraction of hog renal cortex We are also quite certain that when we are able to protect the dogs against death *this protection is on the basis of something in the* nonrenin fraction of hog renal cortex Our group showed eight years ago that antirenin to human renin neutralizes human renin but not the renins of a number of other species We also found that antirenin to hog renin does not neutralize rat renin and does not neutralize human renin

Tremont Smith When you prevent hypertension with antirenin do you give a series of injections before you clamp the renal artery?

Wakerlin Yes You can use any routine you want to produce immunization We have been giving daily intramuscular injections and our dose has varied anywhere from one half unit to as much as ten units of renin per kg of body weight daily We have usually given the injections for three months prior to renal artery constric tion If the titer of antirenin is above three or four units per cc of serum protection is usually obtained

Stamler Can you prevent the malignant vascular lesions in animals with complete ligation of both renal arteries?

Wakerlin We have not tried that We have produced malignant hypertension by the technique that Dr Goldblatt first reported

Stamler Have you done any studies on the changes in the renal circulation as measured by clearances?

Wakerlin Yes two students of mine Drs J M Kiely and J P Kiely found no significant change in the renal clearances of renal hypertensive dogs that were successfully treated with a good anti renin titer and animals that were not protected due to low antirenin titer Likewise in animals that were protected versus animals that were not protected the changes in the renal clearances after con striction were the same in both groups

Last fall our research group(5) reported on what I think is a very crucial experiment that is passive immunization with anti renin So far we have treated eight chronic renal hypertensive dogs with antirenin obtained from other dogs and in general the results are that if the passively produced titer is above the critical level of five antirenin units per cc of serum the hypertension is reduced without any toxic effects and remains down as long as the titer remains up The length of time that the titer remains up is (similar to other antibodies passively administered particularly homo logously prepared antibodies) about two to three weeks The blood pressure remains down for that period and as the antirenin dis appears from the circulation the blood pressure goes up to the previous hypertensive level We hope to extend this series par ticularly with relation to determining what the minimum effective titer is in long standing hypertension as compared with early chronic experimental renal hypertension

Recent experiments may throw some light on the problem that Dr Ogden has raised in regard to a possible change in pathogenesis as experimental renal hypertension continues One of my students Mr E A Ohler found with a new sympatholytic agent which Smith Kline and French have synthesized known as 688 A (N 2 chloroethyl N phenoxyisopropyl benzylamine HCl) that there was no significant reduction in blood pressure in 7 dogs with *early* experimental renal hypertension using early" to mean less than three months whereas there was a significant reduction in 11 dogs with late experimental renal hypertension or hypertension for six months or more Dr W G Moss using less potent sympatholytics

it could have been that the animal remained normotensive partly for that reason namely, the impetus to hypertension was not so great as in our recent experiments

Fremont Smith Do you know whether you protect against VEM, also?

Wakerlin Along with antirenin, anti VEM is also produced Dr Shorr and his associates tested some of our animals To a limited extent we have tested for anti SPS or anti sustained pressor substance, and have found it present in the limited number of animals tested In other words the renins that we have used obviously contain some VEM and some SPS

The correlation with antirenin now that we remove all the medullary protein particularly from crude hog renin is excellent With crude hog renin the antihypertensive results are as good as with semi purified hog renin and I am not altogether convinced that *a purified preparation is the best thing on the basis of our findings* with malignant hypertension where there is obviously some renal protective factor in the non renin fraction of the cortex

Goldblatt On what basis do you say that?

Wakerlin A graduate student Mr R O Burns and I(4) have developed a technique for producing malignant hypertension in nearly 100 percent of dogs with resulting hypertension arterio and arteriolonecrosis and death 100 percent of the dogs treated prophylactically with semi purified hog renin from renal cortex died although they were protected against the hypertension during the period of survival *if the antirenin titer was sufficiently high and frequently* but not regularly against the vascular lesions On the other hand with crude hog renin prepared from renal cortex there was protection against hypertension when the antirenin titer was sufficient protection against the vascular lesions in all of the animals and protection against death in more than half With crude hog renin prepared from whole kidney there was no protection against hyper*tension* against the vascular lesions or against death The evidence suggests that the nonrenin fraction of renal cortex in the absence of medulla protects from death and vascular lesions Accordingly I am not sure whether one is doing the best thing in highly purifying renin from the *standpoint of its therapeutic action in experi*mental renal hypertensions I am reminded of what happened in regard to the purification of liver extract for pernicious anemia when something was lost through too high a degree of purification

antibodies to the structural proteins of the human kidney in addition to the antirenin I caution against use of patients at the present time We plan to work this out in the monkey as thoroughly as possible before we go over to the human

Even though Dr Goldblatt and his group (and our group as well) found that 37 units of antirenin to hog renin did not neutralize one unit of human renin *in vitro* there is still a possibility that the situation *in vivo* may be different Thus we have treated two chronic renal hypertensive monkeys with semipurified hog renin and have produced good titers of antirenin to hog renin (20 and 80 units per cc of serum) without any antihypertensive effect which would fit in with the fact that antirenin to hog renin does not neutralize monkey renin But in prophylaxis experiments on two monkeys one monkey showed what could be interpreted as partial protection with a titer of 15 units per cc of serum and the other monkey with a titer of 25 units was completely protected against hypertension We believe the latter to be a bona fide experiment because we have never failed to produce hypertension in monkeys by constriction of the renal arteries with the technique we use Furthermore the pressure in the treated monkey remained normotensive for three months after constriction and then as the antirenin titer disappeared after injections of hog renin were discontinued the pressure became hypertensive in inverse ratio to the antirenin titer It may be important in such experiments to have all renal medullary protein absent from the hog renin (prepared from renal cortex) Moreover I would not be satisfied with the titer of 40 antirenin units per cc serum in man obtained by Dr Goldblatt's group I think higher titers are possible and that we should keep out renal medullary protein until we have proved by experiments in the dog and in the monkey that it is not important when highly purified hog renin is used

I think it is possible that there may be a significant percentage of patients with essential hypertension who have their hypertension on a renal renin basis One reason for this is our findings in relation to spontaneous (essential?) hypertension in the dog As Dr Katz and his group and others have found so our research group every now and then encounters a dog that we classify as having "essential" or spontaneous hypertension We have treated two such dogs with semipurified hog renin One dog with a titer of antirenin of 120 units per cc of serum showed an excellent reduction in blood pressure to a low normotensive level The other spontaneous hyperten-

in the dog failed to confirm Ogden's findings in the rat I think the early appearance and disappearance of renin in blood might be used to differentiate between early and late experimental renal hypertension We plan to determine what difference, if any there is between the early renal hypertensive and the late hypertensive dog from the standpoint of passive immunization, and also from the standpoint of active immunization with hog renin So far we have treated by active immunization several dogs which have had hypertension for as long as six and seven years with reductions to normotension when a good antirenin titer was obtained, but I can not say whether the minimum effective titer is the same or greater for late as compared with early renal hypertension

We are now interested in producing antirenin to human renin in the horse dog and goat, and are collecting human kidneys The experiment of passively producing an antirenin titer to human renin in man should give us information on the percentage of patients with essential hypertension who have their hypertension primarily on a renal renin basis Our results with passive immunization when sufficient titer was obtained point strongly to the fact that renal hypertensive dogs have their hypertension on a renal renin (or closely related possibly SPS) basis We think the same method should be tried in the human even though the problem is more *difficult since the antirenin must be prepared in another species* We then should be able to tell whether Dr Goldring and his group are right namely that 0 percent of essential hypertensives have their hypertension on a renal basis or whether Dr Goldblatt and his group are right that the percentage is 100 or nearly 100 Possibly it is 50 percent or 30 percent If it should be 10 percent or more from a practical standpoint this type of therapy would be an extremely worthwhile possibility I am not referring to passive immunization with its limited possibilities for therapy but to efforts at altering the antigenicity of hog or other animal renins to make their antirenins effective when human hypertension is on a renal renin basis

As suggested by Dr Helmer we must consider the possibility of producing an experimental (antiserum) glomerulonephritis if we include structural kidney proteins in our human renin preparation Accordingly I caution against using frozen kidneys for renin extraction because we know that frozen and thawed tissues are more likely to contain broken cells than are chilled kidneys Such material would be more likely to produce an antiserum that would have

at the serum farm at Otisville for diphtheria antitoxin and for antipneumococcus serum He later got high levels of antibody for bird plasmodia It would seem to be a technique that ought to be tried out in relation to the antirenin problem

Since your material is not pure renin you get antibodies to other renal factors I still think it is quite possible that the agent for renal hypertension – and I have no doubt that there are in man as in experimental animals renal forms of hypertension – may be humoral but active not on the arteries but solely on the vasomotor center You may be building antibodies for that

Wakerlin We have tested several technics for increasing antirenin production and we are still trying to devise a regimen which will give us maximum antirenin production with minimum amounts of renin Aquaphor as a menstruum has not helped particularly Renin appears more related to the hormone group with which you do not get much increase in antihormone production by the techniques that give increased antibody production with bacterial antigens

Dock You did not try mixing the aquaphor with tubercle bacilli?

Wakerlin We were not acquainted with the Freund technique

Kellner If you use the complete Freund menstruum you generally get a more marked antibody response than by using any one of the agents separately

Grollman I must point out that there is a large body of evidence to indicate that renin or other pressor agents assumed to arise from the kidney play no part in the pathogenesis of chronic hypertensive disease either of the clinical or experimental variety Such evidence if accepted would invalidate many of the assumptions which have been accepted implicitly in this evening s discussion If renin plays no part in the pathogenesis of hypertension it is hardly to be expected that experiments such as those described by Dr Goldblatt would result in a reduction of the blood pressure of his patient Several possibilities may be suggested to explain the apparently beneficial effects obtained by Dr Wakerlin using essentially the same procedure in hypertensive dogs In the first place it is difficult to predict with certainty that a given animal will develop hypertension Hence the use of extracts to prevent the development of hypertension cannot be accepted as definite evidence of their efficacy In the second place lowering of blood pressure by the

sive had an antirenin titer of only 2 and there was no decrease in pressure We are going to repeat the treatment in this latter dog with higher dosage of hog renin in order to obtain a more favorable antirenin titer In other words it is suggested that spontaneous hypertension in the dog may be on a renal renin basis

Also interesting in this connection is the result that we have had in two dogs with neurogenic (buffer nerve) hypertension We treated one with *semipurified hog renin and got a titer of two* antirenin units per cc of serum with no reduction in blood pressure The other dog showed a titer of 40 antirenin units and a significant reduction of blood pressure (*half way* to normotension) This suggests to us that even in neurogenic hypertension in the dog a part of the hypertensive mechanism is on a renal renin basis

In closing if the planned passive immunization experiment with antirenin to human renin should show that a significant percentage of patients with essential hypertension have their hypertension on a renal renin basis then the best that we could hope for from passive immunization therapy would be tiding a patient over an acute hypertensive episode perhaps bringing him out temporarily at least of a malignant episode

Dexter I had hoped to employ human renin therapy on six, eight or ten well chosen cases That would be enough to convince me if the blood pressure fell completely to normal that the human hypertension was on a renal basis

Wakerlin We should try to get the answer by the passive immunization *technic that I have mentioned* with *care to see that* the patients are not subjected to any unusual risk Perhaps it would be easier to work with dog renin in the dog to see if we can modify the antigenicity *of dog renin so that it will give rise to antirenin in the dog* If this could be done with dog renin I would feel encouraged that it could be done with human renin I understand from immunologists that this is a difficult task

Dock Have you considered the technic that immunologists use now of putting antigen *in Freund's menstruum(6) which raises* the titers? This is what Elvin Kabat(7) at Columbia and Isabel Morgan(8) at Johns Hopkins do with encephalitis They take a pinch of monkey brain and add it to a mixture of aquaphor detergent and dead acid fast bacilli One injection of that intramuscularly produces unusually high titers Freund originally used this method

INCIDENCE OF ATHEROSCLEROTIC DISEASE DURING WAR YEARS

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THE MORTALITY from circulatory diseases in Norway showed a marked decrease during the last world war(1). Figure 24 shows the variations from 1927 up to 1948. These changes have been attributed to the food rationing which in Norway was rather severe and experiences from other countries may add support to this view.

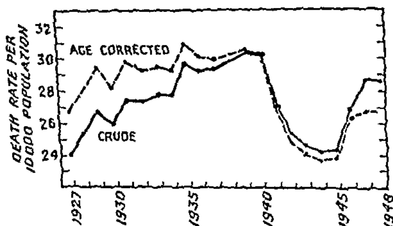


FIGURE 24. Mortality from circulatory diseases in Norway in 1927-48. Standard population = population of Norway in 1940. Reprinted from article by Strom and Jensen, *Lancet* 260: 127 (1951).

TABLE IX

Mortality from Circulatory Disease + Apoplexy and Chronic Nephritis in 1941-47 Related to Mortality in 1938-40 Which is Expressed as 100. (Standardized Death Rates Standard Population: Civilian Population in England and Wales (1938))

	1938/40	1941	1942	1943	1944	1945	1946	1947
England	100.0	94.5	89.5	87.2	89.8	89.7	91.6	97.1
Denmark	100.0	99.6	94.4	90.4	93.3	100.4	110.8	110.2
Norway	100.0	88.8	81.4	78.0	77.1	78.8	85.5	87.1

parenteral administration of relatively crude heterologous protein solutions may always be attributed to the non specific noxious influence of such material

With evidence now available to indicate that renin may not play a major role in the pathogenesis of hypertension, I can not share the enthusiasm of the speakers for the view that the solution of the problem of hypertension lies in anti renin

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THE MORTALITY from circulatory diseases in Norway showed a marked decrease during the last world war(1) Figure 24 shows the variations from 1927 up to 1948 These changes have been attributed to the food rationing which in Norway was rather severe and experiences from other countries may add support to this view

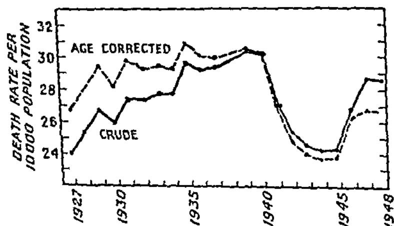


FIGURE 24 Mortality from circulatory diseases in Norway in 1927-48 Standard population = population of Norway in 1940 Reprinted from article by Strom and Jensen *Lancet* 260 1?? (1951)

TABLE IX

Mortality from Circulatory Disease + Apoplexy and Chronic Nephritis in 1941-47 Related to Mortality in 1938-40 Which is Expressed as 100 (Standardized Death Rates Standard Population Civilian Population in England and Wales (1938)

	1938/40	1941	1942	1943	1944	1945	1946	1947
England	100 0	94 5	89 5	89 2	89 8	89 7	91 6	97 1
Denmark	100 0	99 6	94 4	90 4	93 3	100 4	110 8	110 2
Norway	100 0	88 8	81 4	78 0	77 1	76 8	85 5	97 1

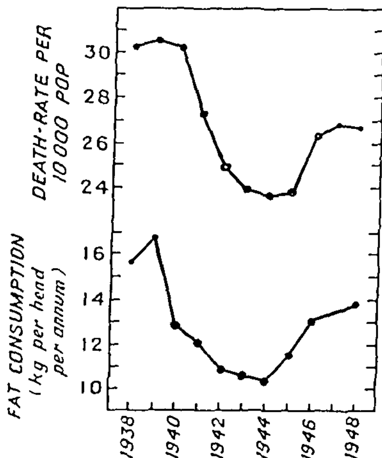


FIGURE 25 Mortality from circulatory diseases corrected for age consumption of fat in form of butter milk cheese and eggs Reprinted from article by Strom and Jensen *Lancet* **260** *128 (1951)*

Figure 25 shows the variation in consumption of different kinds of food There was a definite decrease in the calorie intake from 3470 to 2849 per day the most drastic reduction took place in consumption of fats from 159 to 71 grams per day proteins were reduced from 115 to 89 grams per day

During the hunger blockade of Leningrad in 1941 42 a marked reduction was found in the incidence of hypertension angina pectoris and coronary occlusion We have no similar statistical reports from Norway neither can we of course speak of hunger in connection with the food rationing although it was a most distressing and troublesome experience We have however some statistical data from the health work in some of our larger factories The employees are checked once a year and variations in blood pressure and percentage of overweight noted The variations are

rather small although statisticilly significant but it is difficult to explain how such changes can have a marked and immediate influence on the mortality from cardiovascular diseases

In the discussion of the pathogenesis of arteriosclerotic disease the theories of Winternitz have been put in the background for the last years We had some I must say rather vague indications for assuming that the coagulability of the blood was influenced by the content of fat in the diet and we tried to elucidate whether food rationing could have any influence on the incidence of thrombo embolic phenomena during the war This could have some bearing upon the validity of the Winternitz theory These investigations(2) are not completed as yet but Figure 26 shows the data from one

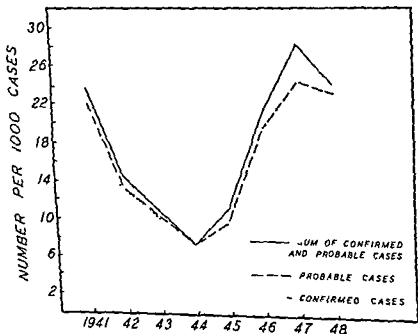


FIGURE 26 Occurrence of thrombo-embolic phenomena per 1000 operations

of our largest surgical wards The dots show the occurrence of thrombo embolic phenomena per 1000 operations the broken line gives the sum of the certain and almost certain cases whereas the solid line gives the sum of cases where the diagnosis of thrombo emboli was conceivable I have just received the last report from another surgical hospital which gives the following figures (Table X)

TABLE X

Occurrence of Thrombo Embolic Phenomena Following Operations in Which Such Complications Commonly Occur (Aker Sykehus, Oslo)*

1940	1941	1942	1943	1944	1945	1946	1947	1948
57.3%	23.6	9.9	15.6	15.4	17.9	18.6	28.5	34.0

The variations show the same pattern. In fact, despite our more modern treatment of surgical cases in recent years (patients are taken out of bed the day after operation and so forth) the incidence of thrombo embolic disease has actually increased.

We have also tried to curtail experimental arteriosclerosis in animals by giving them anticoagulants(3). The first series of experiments on 18 cockerels which were implanted with stilbestrol according to the common technique were rather encouraging with very little arteriosclerosis as compared to the controls.

The coagulability of the blood in the untreated chickens showed a good correlation with the degree of lipemia (based on cholesterol estimations). We found more rapid coagulation with higher cholesterol levels. The same changes could be found after injection of stilbestrol and were confirmed when we used the two stage prothrombin estimation test of Owren as shown in Figure 27.

We repeated our experiment in rabbits. Although some animals showed a good correlation between prothrombin concentration and cholesterol and lipid content of the blood, we could not find the same consistent correlations as in chickens. Whether this is due to the difference in the composition of the lipids in the blood in the two animals, with high phospholipids in the chickens in contrast to the low levels in rabbits, we do not know. We also were unable to demonstrate any effect of dicoumarol on the arteriosclerotic process in these animals.

Shorr Did you obtain serum calcium values at the same time?

Dedichen No, we have not. I do not think the calcium would influence those results.

Shorr But serum calcium will go up with the administration of stilbestrol.

* R. Adelsten Jensen. Unpublished.

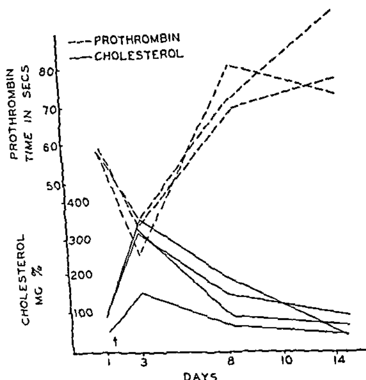


FIGURE 27 Variation in prothrombin time and cholesterol after stilbestrol injection in cockerels

Dedichen Probably it will but I think in the two stage method using diluted plasma this will not influence the result at all

Dock The dicoumarol protection is less striking in the rabbits?

Dedichen Very much I think it is difficult to get dicoumarol protection in rabbits but it is quite obvious in chicks

Goldblatt Have you tried heparin?

Dedichen We have tried P I D not heparin due to technical difficulties

Katz Have you compared your results with those of Malmros of Denmark?

Dedichen Dr Malmros results are comparable but they are not I think as conclusive being based upon selected statistics from hospital records Our statistics are from death rates in the health records

Cofman Graham in our laboratory has been doing some experiments on the effect of certain anticoagulants The observation had been reported some time ago that visibly lipemic serum can be cleared by the use of heparin given parenterally Graham has shown in the human that heparin is one of the most active agents we have yet seen for the production of acute changes in the blood lipoprotein picture Molecules of the 30 to 100 S_f classes, and all the way up to chylomicrons can be essentially wiped out in a large percentage of human subjects with a single injection of 50 mg of heparin, within a period of 15 minutes to an hour During the next period of about an hour there is a progressive disappearance although there may be a transitory increase in the molecules of the 20 to 30 S_f class and even the 10 to 20 S_f class The lower S_f classes increase at the apparent expense of decrease in the higher classes

This perhaps supplements Dr Dedichen s remarks on the effects of high fat diet on increased coagulability It is interesting that heparin rapidly depletes that portion of lipemia which might best be described as the 30 to 100 S_f molecules This class is greatly altered by fatty meals in many but not all people

Ogden How long does that last?

Cofman The effect on the lipoprotein molecules apparently lasts longer than the usually ascribed anti coagulant effect of heparin and is still noticeable at 24 hours although by this time after a single injection of regular aqueous heparin the picture is returning toward normal We have some patients who are now receiving heparin experimentally once a week in an effort to find the duration of effect

Kellner Did you study lipemic rabbits or human subjects? We were interested in the observation by Hahn(4) in dogs that heparin in a matter of minutes abolished visible lipemia We tried it in rabbits using turbidimetric methods for measuring the lipemia and found that heparin given intravenously had no effect on the lipemia

Gofman In rabbits the effect may be different from that in human *subjects* although *some* rabbits show the same general effect

Kellner I should like to ask Dr Dedichen whether he has any suggestions as to the mechanism whereby the prothrombin time

is decreased and the blood becomes more coagulable during lipemia The work of Tocantins and Overman indicates that there is a physiological antithromboplastin in humans which appears to be a lipoprotein If all the lipids in the serum were increased in a non specific fashion one would expect that the antithromboplastins would also be increased and the prothrombin time therefore pro longed

Dedichen We thought at first that it could be a mere physical phenomenon that it was much easier to see the starting of the coagulation process in plasma containing much lipid After having checked our results with the two stage method we thought we could rule that out

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CARDIOVASCULAR DISEASE IN CEYLON

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I HAVE WONDERED what would have happened if modern physiology had started in the East and not in the West, because we should then be wondering why the people who live in the West had not gotten an eosinophil count of at least 15 percent and why they did not all harbor worms and parasites in their intestine, why they had vital capacities greater than 2.5 liters. We hardly ever see anybody in Ceylon with a vital capacity greater than 2.5 liters or so. These things were noticeable to me when I went to Ceylon four and a half years ago where the students are taught from Western textbooks.

I also discovered that one of the common conditions that had been diagnosed in western Ceylon was hypotension. The well to do Ceylonese who did not feel too well went to a physician and had his blood pressure taken. He was told, "Well, you have a low blood pressure," and he was perfectly satisfied with that.

We set up as one of our research projects — it has been going on now for four years — a series of surveys of the whole nation of Ceylon. We got samples given to us by the Department of Statistics. We went around the whole island and religiously measured all kinds of things on the people living in different environments in Ceylon. We have measured the blood pressure in about 20,000 people. The blood pressure of healthy male Ceylonese aged 20 to 25 is 105 systolic and 66 diastolic. When expressed as modal blood pressures the figures are lower, 103 and 62. This blood pressure does not rise with age much above 110 systolic or much above 70 diastolic. There is no influence of race on the blood pressure record-ings. There are several races which are indigenous. There are the Sinhalese, Tamils, Moors, Malays, and descendants from the old Dutch colonists. The Dutch ruled Ceylon for one hundred and fifty years and wherever they colonized they always intermarried with the local people. Therefore we have Dutch burghers with a lot of European blood in them. All these different ethnic groups, as the sociologists now prefer to call them, have indistinguishable

systolic blood pressures There is no influence apparently, of social or economic status

One thing which apparently does affect blood pressure in Ceylon is altitude The higher you live in Ceylon the lower your systolic blood pressure People living about 6000 feet above sea level have a systolic blood pressure which averages about 10 mm less than those living at Colombo at sea level You can reproduce this fall in blood pressure by taking subjects as we have done, up to various altitudes and letting them acclimatize

There are other alterations at moderate altitudes such as a lowering in the blood volume and plasma volume and a lowering in the interstitial volume An increase in the lymphocyte count occurs There are many alterations with moderate altitude which apparently have not been recognized up to the present moment because we have been chiefly concerned with much higher altitudes than six or seven thousand feet

Dock Does the average temperature go up or down as you get away from the sea?

Cullumbine The average temperature throughout the year will be lower at altitude than down at sea level There is a fall of about 1 F for each 300 feet rise in altitude We were careful to do our so called experiments with subjects of like climate so that we had them at altitudes at similar temperatures and we have been able to reproduce these findings in animals exposed to low barometric pressure

The apparent incidence of deaths from cardiovascular disease is extremely small Whereas in America out of every thousand deaths perhaps 340 die from cardiovascular disease in Ceylon out of every thousand deaths only 20 or 30 die from cardiovascular disease

There is a racial difference in incidence of cardiovascular disease The Dutch burghers are largely responsible for the incidence of deaths from cardiovascular disease The more indigenous races the Sinhalese and the Malays and Tamils don t die as readily from cardiovascular thrombosis as do the burghers

Economic status is very important We find the higher the economic status of the people the greater the death rate from cardiovascular disease

As for city life, we have only one city that is comparable to anything you have in the West, and that is Colombo, which has a population of 800 000 The incidence of deaths from cardio vascular diseases is far higher in Colombo than in any other part of Ceylon

One reason why cardiovascular disease is not very common in Ceylon is possibly that the life expectation in Ceylon has been very small In 1946 the life expectation for males was 44 years for females it was 42 Since 1946 they have had an ambitious program of DDT residual spraying and they have eradicated malaria more or less in Ceylon So that by 1949 the life expectation for males had gone up from 44 to 54 Thus in three years the life expectation has been increased by ten years For females it had gone up from 42 to 53

Along with this increase in life expectation the incidence of cardiovascular disease had also increased There had been about a 50 percent increase in incidence of deaths from cardiovascular disease and in hospital morbidity from cardiovascular disease

If we compare the vital *statistics for Ceylon with those* of a small agricultural nation in the West and for that I have taken Eire southern Ireland we find in comparable age groups that cardiovascular disease has only a tenth of the incidence as judged by vital statistics and hospital morbidity in Ceylon compared to Eire Again that may not be a fair comparison The people who live long enough in Ceylon to acquire cardiovascular disease are a selected population It has been survival of the fittest in the past

We have done a dietary survey which has covered the whole island of Ceylon The fat intake is very small We have to judge this again of course according to the economic status of the people We recognize three economic levels in Ceylon for the purpose of our survey People living at the lowest economic level take about 30 gm of fat a day on the average the intermediate level the clerks and people like that have a fat intake of about 50 gm per day and the higher economic group the professional and businessmen take about 90 gm of fat a day That fat intake variation *corresponds to the variation in death rate* again from cardiovascular disease if you like to look at it that way

We cannot give you much information about blood cholesterol So far we have only characterized the children in Ceylon from the point of view of *cholesterol content in their blood* Our average

figure is 149 mg percent total cholesterol with a range between 103 and 203 That is for several hundred children aged between 4 years and 7 years Their blood lipids averaged 15 8 and ranged between 12 8 to 19 3 I don t know how they compare

Dock For the age group I think they are quite comparable

Kellner I think too they are quite comparable

Cullumbine For prothrombin time my method has been the Quick one stage method which should give us a reading some where around 11 12 seconds We have never yet gotten such a value in Ceylon when faithfully imitating this method but we invariably get a prothrombin time of 18 or 19 seconds unless the patient has some disease condition which will alter it

The total caloric consumption of course in Ceylon is low We have very low basal metabolic rates which are not due I think to persistent undernourishment because if you take well nourished people from high economic groups they still have extremely low metabolic rates

Shorr How much lower are the metabolic rates than in this part of the world?

Cullumbine A man weighing about 70 kilos in the West you would say would need about 70 calories per hour Our men would need somewhere around 48 calories per hour

Shorr As you may know revaluation of the Aub Dubois standards derived from a large series of cases suggests that they should be corrected by about 8 percent i e they are 8 percent too high

Cullumbine This is more than the 8 percent correction

Wakerlin May I ask what are the principal causes of death in those who die between the ages of 55 and 65 or 70?

Cullumbine A few people die from cardiovascular disease a few people die from cancer a few people die from results of peptic ulceration there are a few people who die from genitourinary diseases There is no major pathology distinguishable as far as we can see in the older age group at the present moment

Goldblatt According to your statistics you would have to call 65 extreme old age

Cullumbine Yes that is true

Dock Is cirrhosis of the liver common there?

Cullumbine It is relatively common We do have also in children malnourishment with a fatty liver, which is called kwashiorkor in Africa and which seems to exist throughout the tropical belt

Kellner How common is diabetes in Ceylon?

Cullumbine It is an extremely rare condition It occurs practically without exception in the Dutch burghers The next problem to tackle in Ceylon, now that we have gotten rid of malaria is that of gastrointestinal infections and infestations, which is a far more costly business than just spraying with DDT

Wilens Is the incidence of tuberculosis very high in Ceylon?

Cullumbine The incidence officially is only about a tenth of that in the West On the other hand a survey was done in a suburban area of Colombo which showed the incidence was probably just as high in Ceylon if not higher than in the West

Goldblatt Is there a low protein intake?

Cullumbine Yes we have a low animal protein intake The vegetable protein intake is fairly high

Perera What about rheumatic heart disease?

Cullumbine Someone said that never occurred in the tropics but it certainly occurs in Ceylon

Goldblatt Thank you Dr Cullumbine We are certainly grateful to you and to Dr Dedichen for participating in the program

BLOOD FLOW, BLOOD PRESSURE AND INTIMAL THICKNESS AS FACTORS IN LOCALIZING ATHEROMA FORMATION

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THE CLASSICAL German pathology textbooks on atherosclerosis be fore 1930 do not mention cholesterol metabolism or fat The dis ease was believed to involve the whole wall of the blood vessel which underwent changes of a degenerative sort and secondarily as in many other degenerative diseases lipid became deposited in this altered tissue

There are at present various theories Dr Rinehart s group(1) at the University of California believes that arteriosclerosis is secondary to water soluble vitamin deficiencies injuring the wall of the artery and leading to degenerative changes Dr Lansing will discuss the effect of medial changes on atherosclerosis Another point of view was that suggested by Dr Simms yesterday Normal fibroblasts in the intima of arteries may take up lipid from altered plasma and this lipid may be secondarily released by the cells and get into the interstitial tissue Finally there is what I regard as the delta theory of atherosclerosis which considers the deposition of lipid in the walls of vessels as something like the deposition of sediment at a place where a stream comes out into a shallow lake or arm of the sea and it is with this variant that I propose to deal today

If for working purposes we turn our attention to this theory the factors that would be important are these in the first place the percolation pressure which forces plasma filtrate into the intima Our data from human pathology would suggest that the threshold level is about 60 mm Hg Dr Wilens has suggested that an atheroma is probably secondary to thrombotic injury to the vein wall At any rate in the veins where pressure is much lower than 60 mm Hg atherosclerosis is either rare or unknown In the pul monary artery where the pressure is normally lower than 60 mm Hg atherosclerosis is also unknown On the other hand in pulmonic

hypertension pulmonary atherosclerosis occurs and may exceed the atherosclerosis in the systemic vessels even in young people

The ratio of cholesterol to phospholipids may be an important factor Dr Kendall has shown that the threshold for atherosclerosis in terms of the ratio of cholesterol to phospholipids was the same in three species, namely the rabbit the dog and man although the blood cholesterol levels are very different in the three species at the time when they begin to develop atherosclerosis

One other important question would be the thickness of the *intimal cushion which might be the innate cushion of the vessel* if it were an anatomically normal feature or the thickenings that occur secondarily either to medial or adventitial thickness

The velocity of blood flow should be included The more rapid the blood flow is the more likely it is for atherosclerosis to develop

The pressure factor is very striking in atherosclerosis of the upper and lower extremities and of the upper and lower part of the aorta In part of the aorta atherosclerosis is uncommon in any advanced form except in the presence of syphilitic medial otitis On the other hand near the bifurcation of the aorta atherosclerosis is quite common and in older people often very far advanced with ulceration

Hamilton s curves(2) for dogs show that as the pulse wave goes down the brachial systolic pressure and the femoral systolic pressure get higher In man the femoral systolic pressure is higher than the brachial systolic pressure when we are lying down The beat of the blood against the vessel wall below the diaphragm is greater than above it When we are erect there is additional hydrostatic pressure so that the pressure in the lower aorta and legs is usually higher than that in the brachial arteries and the vessels above *the diaphragm The exact levels of these would* depend upon the structure and elasticity of the aorta There are differences in this respect between dogs and man

The femoral pressure in man lying down is higher than in the brachial When we are standing up the pressure *is much greater* That suggests that some of the anatomic differences in the severity of atherosclerosis may be related to differences in pressure

Dr Wilens(3) has published a very ingenious study of cerebral arterial hydrodynamics in relation to the *suction of the cerebro* spinal fluid when we are sitting up and showing some correlations

between body structure and the relative frequency of cerebral and of coronary atherosclerosis He suggested that excessive pressure in the cerebral arteries in relation to the pressure existing in the cerebrospinal fluid is a factor in predisposing to atherosclerosis there The level of pressure in the arteries perhaps contributes to the rate at which atherosclerosis will develop when the blood plasma has a cholesterol/phospholipid ratio which is above the threshold that we have already mentioned The factor of pressure however cannot explain all of the localizations that we see in atherosclerosis and particularly it won t explain why atherosclerosis is so common in the coronary arteries certainly there is no hydrostatic factor there

An important consideration in hypertensive men who develop atherosclerosis is that the disease occurs out in the arterioles and in the small arteries where we practically never see it in normotensive people developing atherosclerosis Levels above the threshold of about 60 mm Hg continue much farther out into the vascular bed in a hypertensive person than they will in a normotensive person

In addition the pressure probably goes much farther out into the vascular bed in those regions where the basal rates of flow are normally quite high This involves the brain the retina and the viscera where the variations in blood flow are much less than they are in the skin in the heart muscle or in the voluntary muscles The skin the heart muscle and the voluntary muscles usually have considerable vasoconstriction to protect them in contrast to the viscera where this is absent This may account for the extension of atherosclerosis so far out into the small vessels of the retina the kidney the adrenal the pancreas and its almost complete absence in the small arteries and arterioles of the heart muscle or the voluntary muscles or the skin

I think we can account then for the involvement of small vessels on a pressure basis and I want to emphasize again that the changes seen in these small vessels are atherosclerotic changes We have Kodachromes of kidneys stained for fat showing atheroma in the arterioles right up to the glomerulus in an ordinary case of hypertensive cardiovascular disease with retinal and visceral lesions of the characteristic sort These are atheroma and they have lipid in them

The pressure hypothesis cannot explain either the spotty distribution of atheroma in a segment of a vessel or the high incidence of atherosclerosis in the coronary artery These can be largely explained on the basis of intimal thickening Apparently the deposition of cholesterol and neutral fat occurs along very fine fibers which are present in the intima, even where this layer is very thin The thicker the intima and the more fibers there are the greater chance there is for this type of deposition to occur

The intima of the coronary arteries is different from that of any of the mesenteric vessels the radials or the tibial arteries Relatively few arteries show intimal thickening normally from birth to advanced old age comparable to that seen in the epicardial parts of the coronary system Spalteholtz s paper(4) which was published some 19 years ago shows the intima and the adventitia of a 6 year old boy Elastic tissue stains show the dark fibers and thickening of the intima the media having a pale stain In places the intima is thicker than the media Similar illustrations had been published in the von Moellendorf(5) encyclopedia of histology

Spalteholtz has shown in this study that there are longitudinal muscle fibers as well as elastic fibers in the coronary intima of human beings There are few capillaries if any in the intima It seems to get most of its nutrition from the lumen of the vessel rather than from intimal vascularization There are however species that have much thicker intimas than this in the aorta and in the coronary arteries Herbivorous mammals have thick intimas which have quite a heavy vasculature These animals never show atheroma

I once sent Dr Winternitz the aorta and the pulmonary and main coronary arteries of an ancient bull elephant which had been autopsied by Dr K F Meyer in San Francisco Neither atherosclerosis nor traces of old intimal hemorrhages were evident in the aorta in spite of a tremendously thickened intima Thus thickening of the intima with a tremendous layer of fibrous and elastic tissue does not necessarily produce atherosclerosis even in old age

We made sections from the epicardial branches of a 20 year old man who committed suicide and found intimal cushions which were as thick as the media Dr Lansing tells me there is no sex difference in the intimal thickening of the coronary in men and women and/or infants although we had some statistics showing that there was Dr Holman of the University of Southern California, was not able to confirm sex difference in newborn infants

However in one newborn female infant all we could see was the inner elastic membrane lined with mesothelial cells There was no intimal cushion in the sense that we use that term no piling up of elastic or argentophil fibers On the other hand we have photographs of the left coronary artery from a male infant showing that the intima at birth was thicker than the media in several places in the artery

I am convinced that the predisposition of the coronary arteries to athero clerosis is related to the thick intima which is present from birth on in this anatomical situation There may not be any anatomical sex differences The sex difference in atherosclerosis must then be attributed to factors such as those suggested by Dr Gofman I might say that our views on the sex difference were confirmed by Dr Hellwig(6) a pathologist in Kansas and by Minkowski(7) in New York

Wilens I have measured the thickness of the intima in a large series of aortas and can confirm Dr Dock s findings in the coronary artery Even in childhood the male aortic intima is thicker than that of the female

Dock A typical section from a coronary artery of a soldier who d ed of general coronary atherosclerosis shows a large xanthoma in the intima although the media under that intima is still quite good in places In other parts the media has undergone the atrophy that regularly occurs with atheroma of the coronary arteries Apparently the disea e in these young men had progressed so rapidly as to cause death without changes in the elastic membrane under the thick coronary intimal masses of cholesterol and fat and newly formed granulation tissue Howe er at the Army Institute of Pathology there are specimens of coronary arteries where the intima is twice as thick as the media with no atherosclerosis We could find no foam cells and no accumulation of lipid in this thickened intima The thickening of the coronary artery *intima is* confined to the branches that lie in the epithelial fat and does *not* involve the branches that penetrate into the muscle Atherosclerosis occurs early and severely in the epicardial branches and occurs rarely and late in the branches that penetrate into the muscle The correlation between the intimal thickening and a *predisposition* to atherosclerosis is especially striking in the coronary arteries

Elsewhere in the vascular bed *thickening of the* intima occurs normally In the places where atheroma occurs around the *inter*

costal branches, *normally there is a thickening of the intima Such* anatomical variations in the thickness of the intima may be important in explaining the spotty distribution of atheroma in the *blood vessels*

In the arteries around the base of the brain I think there is another factor of some importance, which brings us not to innate intimal thickening but to secondary intimal thickening Whenever the media is diseased, as in syphilitic mesaortitis, the intima thickens In the cerebral arteries, adventitial weakness is an innate disorder which in advanced stages is seen in aneurysms of the circle of Willis In many places these arteries have a thin adventitia When one injects solidified lead carbonate jelly at 100 mm Hg pressure in the coronary arteries bulges do not appear at the point where there is atheroma while in the cerebral circulation they do appear The atheroma occurs at places where the adventitia is weak and where there has been a bulging out of the wall of the vessel Intimal thickening can occur without atheroma or there may be atheroma where the intima may appear to be weak

Gutman Is there any sex difference in the cerebral artery adventitia?

Dock I have never done such studies but they should be done by injection under arterial pressure fixing the wall at a pressure of about 100 mm Hg Secondary intimal thickening due to syphilitic aortitis to arteritis to degenerative changes in the media or to inherited weakness of the adventitia seems to us then to be very important

In addition to these changes in the intima innate or secondary I want to emphasize again velocity of flow One of the most striking pathological observations is seen in a patient who has had long standing arteriovenous fistula in the leg The atherosclerosis in the iliac artery going to the fistula is far more severe than in the iliac artery going to the good leg The pressure must be lower in that artery because the blood is leaking out of that side so it is not due to increased pressure It can only be related to an increased velocity of flow Increased velocity of flow should cause an increased vibration in the wall of any vessel

There is one other place where an atheroma forms which seems to me to be a site of intense vibration In many of the young soldiers who die with coronary disease the only other atheroma we see is in the anterior mitral leaflet The posterior leaflet shows

nothing but in the anterior leaflet atheroma is quite common The anterior leaflet is not damped the way the posterior is, being up against muscle It hangs free in the center of the chamber It is inserted at its base into the root of the aorta, and it shakes violently We think that vibration in vessel walls with velocity of flow as a cause of that is related to the speed with which atheroma develops that the thickness of intima and the level of pressure in the arteries are also important Accordingly atherosclerosis would be regarded as a result of many factors some inherited and some acquired

Katz Would you accept the alternative that velocity of flow might act as a frictional irritant causing focal thickening?

Dock That is quite possible

Goldblatt In all those cases in which you demonstrated considerable thickness of the intima of the coronary or other vessel had you eliminated rheumatic heart disease?

Dock We thought we had very thoroughly

Major French Colonel Lucke and I considered that point carefully at the time Other pathologists have reexamined these cases and believe that all these soldiers had arteritis Similar lesions were rare in British soldier who had rheumatic disease but were on a different diet

I think it is unlikely that Dr von Moellendorf in Switzerland and Dr Spalteholtz in Germany could not tell whether an arterial wall had been inflamed It was pointed out by von Moellendorf that this is not peculiar to the coronary arteries since it also occurs in other arteries that have a change in their length One germane illustration is the occipital artery which stretches and shortens as you turn your head from side to side These vessels have intimal cushions just the way the coronary does The arteries in the corpus *cavernosum* show this even more strikingly This anatomical feature apparently is common to arteries that change their length greatly in the course of their physiological variations Of course the epicardial branch of the coronary changes length with each heart beat This is a *normal* innate anatomical feature of certain arteries Atheroma can be seen in small arteries of the kidney when the vessels have been injected with lead carbonate before fixation Without such *preparation* none of these vessels show any lumen After injection atheroma are visible in the smaller arteries with extremely thin walls The wall may be a few micra

thick where it is not diseased, but about 30 micra thick where the atheroma has formed

Barr How big are the vessels?

Dock The arterioles are about 40 microns in diameter

Wilens Do you think that the lipid is entirely in the intima of the arterioles?

Dock Yes, it is all in the intima which appears to have thickened and become full of hyaline material When you stain the sections with H & E, the presence of eosinophilic material is indicated The thickening there consists of protein which stains in an eosinophilic manner with H & E, and in a Sudan stain it looks as though it were all fat Probably it is a fine emulsion of lipid in protein or a lipoprotein complex

Kellner Did you study the arterioles in the spleen that undergo hyaline change with age and did they contain lipid?

Dock In this type of patient they do In a patient with minimal atherosclerosis and who is not hypertensive there is very little lipid in the splenic vessels

Barr Did you study any young diabetic with that technic?

Wilens I have done fat stains on kidneys of diabetics at various ages and have found lipid in the arterioles of 67 5 percent of those with associated hypertension and in 41 2 percent of those without associated hypertension(8)

Dock Did you inject the kidneys?

Wilens No I did not I have no doubt however that I was able to demonstrate the same lesions that you have just shown There are several features about this lesion that are different from arterial atherosclerosis The lipid is not anisotropic and is never found within foam phagocytes Moreover the lipid seems to be diffused throughout the entire wall of the arteriole and not just limited to the intima as in arterial atherosclerosis Furthermore there is no real proof that the arteriolar lipid contains cholesterol Recently however Baker and Kent(9) concluded from differential staining techniques that the arteriolar lipid deposits did contain cholesterol

Dock I believe it may be cholesterol in a very fine form Cholesterol is not anisotropic until it occurs as large granules

Barr I wonder whether Dr Wilens noticed any particular distribution of lesions in young diabetics

Wilens The diabetics that I studied were not young They consisted of a group of 20 diabetics without hypertension and 20 with hypertension A few of these were from 20 to 30 years of age Lipid was found in the afferent arterioles and even in the intraglomerular capillary loops(8)

Barr Was the lesion that you found in proximal arterioles or arteries similar to that which you found in the intraglomerular loops?

Wilens It was essentially the same except that in the group with both diabetes and hypertension the lipid was often in spherical masses similar in shape and position to the hyaline masses that are considered to be characteristic of intercapillary glomerulosclerosis

Goldblatt In those cases Dr Wilens did the picture with the ordinary stain look like this so called intercapillary glomerulosclerosis that frequently occurs in diabetes or did you think it was different?

Wilens Initially we had noted in several cases of intercapillary glomerulosclerosis that the glomeruli contained excessive amounts of lipid often in the form of spheres From our further studies we concluded that the hyaline spheres may originate as spherical globules of lipid that are later converted into hyaline material(10)

Goldblatt You believe then that the process in the glomerulus is like that in the arteries a manifestation of the same process?

Wilens Yes I do

Lansing When questioned earlier regarding the sex difference in the human coronary artery I was referring only to newborn and stillborn cases I agree with you Dr Dock as regards the sex difference in six seven and eight year old children and also as regards the capacity of the coronary to become hyperplastic at such an early age The coronary differs from other vessels in that the changes in the intima at least as regards hyperplasia or fibrosis occur at least a decade and probably two decades earlier than in arteries such as the renal hepatic iliac and the aorta I wonder whether you agree with me then that there must be a thickened intima in order to get cholesterol accumulation?

Dock I think the rate of cholesterol accumulation atheroma formation will be most rapid where the intima is thickest However the vessels in the kidneys have practically no intima, yet they become atherosclerotic in certain diseased states With silver stains one can demonstrate a few intimal fibrillae for there are argentophil fibers between the mesothelial cells and the muscle cells Thus there is a structural framework on which cholesterol could be deposited In the coronary this framework is abundant I regard the intimal thickening in the coronary as normal and not hyperplastic

Lansing I have never seen in several hundred cases that we have examined at any age, a thickened intima without underlying medial tissue changes involving fraying of the elastic tissue

Dock This will all depend on a matter of definition with respect to what you regard as fraying of the elastic tissue

Goldblatt Dr Lansing do you assert that thickening of the intima is a necessary condition for the deposition of lipid material in the intima?

Lansing I think I have to answer yes All my statements are based on observations in the human arterial system

Goldblatt There is another way of interpreting that that the thickening is merely a reaction to the presence of lipid deposits

Lansing *Possibly*

Simms Dr Lansing have you gone down to the tiny beginning nodules where there is just a beginning deposition of fat and made observations as to the thickening there?

Lansing Not analytically no

Stamler Dr Leary s slides of human beings with intimal foam cell cushions show no evidence of preliminary thickening that I can see

I want to offer one other comment We have a number of slides similar to those which Dr Dock showed of renal arterioles from chicks on high cholesterol diets Moreover the small coronary arteries and intramyocardial arterioles show a very *similar pattern* in such chicks

Wilens Arteriolar lipid deposits in rabbits fed cholesterol were first noted many years ago Anitschkow described them in the follicular arterioles of the spleen in 1914(11)

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CHANGES IN THE MEDIA IN HUMAN ARTERIOSCLEROSIS AND HYPERTENSION*

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THE REMARKS I want to make are entirely those of a chemical morphologist who actually is offering little that is new to the literature. We have used several new techniques in describing the events of arteriosclerosis but on the whole comparable observations are already in the literature. They are brought together quite effectively in Cowdry's *Arteriosclerosis* (1) which was published by the Macy Foundation in 1933. The consensus at that time, when cholesterol experiments were very new, was that changes were occurring in the media of the artery which could be correlated with the changes in the intima.

I do not intend to minimize the role of cholesterol metabolism in arteriosclerosis. One has only to examine a plaque containing large aggregates of cholesterol to visualize the importance of this material. On the other hand, if one attributes the genesis of arteriosclerotic lesions entirely to faulty cholesterol metabolism a number of paradoxical situations immediately arise. The cholesterol concept does not explain the fact that some plaques are entirely fibrous and do not contain cholesterol; it does not explain the focal nature of arteriosclerotic lesions, the greater severity of lesions in the abdominal than in the thoracic aorta, and the relative resistance of some arteries, such as the pulmonary, to plaque formation. Nor does it explain the relation between cholesterol metabolism and luetic aortitis or mitral stenosis, both of which characteristically are associated with severe arteriosclerosis.

If, however, one takes into account significant age changes in the architecture of the arterial wall it becomes possible to rationalize these foregoing anomalies. Our observations on arteriosclerosis indicate two things: first, that there are changes in the media which

* These studies have been supported by grants from the American Heart Association and the U. S. Public Health Service.
Most of the original work reported herein has been previously published jointly with T. B. Rosenthal, Research Associate in Anatomy, and M. Alex, Research Assistant in Anatomy, Washington University School of Medicine.

can be correlated with changes in the intima and secondly that it seems most likely that these *medial* changes precede intimal plaque formation Perhaps it would be more accurate to say that the intimal thickening forms or exists as an excellent nidus for cholesterol accumulation This is a common relation but there may be *situations in* which cholesterol accumulates directly in the non thickened intima

To further retrace our steps several characteristic lesions are generally associated with arteriosclerosis but not too well related to the disease Karsner takes one point of view Moore is cautious and does not attempt to relate intimal fibrosis to the fatty intimal plaque the ulcerating necrotic fatty plaque to the calcified plaque or Moenckeberg's sclerosis of the muscular artery to the elastic tissue changes of the elastic arteries There is still another *intimal* lesion the calcareous or ossified plate the one frequently seen in older aortas with no apparent cholesterol accumulation



Figure 28a Newborn infant aorta Verhoeff elastic tissue stain x 50



FIGURE 28b Darkfield photomicrograph of microincinerated preparation of new born infant aorta x 90

We have then essentially six atherosclerotic lesions four of them being intimal the fibrosis the fatty plaque the necrotic and calci fied plaque the calcareous plate or ossified plate two are medial lesions the vague pattern of elastic tissue changes in elastic arteries and the Moenckeberg s sclerosis the calcareous mass in muscular arteries I think these are all part of a closely related series of age conditioned changes I doubt that Moenckeberg s sclerosis is an isolated event it is directly connected with the elastic tissue changes in the media of the muscular artery and is analogous to the changes in elastic arteries

With that preamble I should like to describe some of the age changes that we have found in the media of elastic arteries

Figure 28a compares a human aorta stained with Verhoeff elastic tissue stain with an adjacent section prepared by micro incineration Microincineration deposits the minerals that are pres

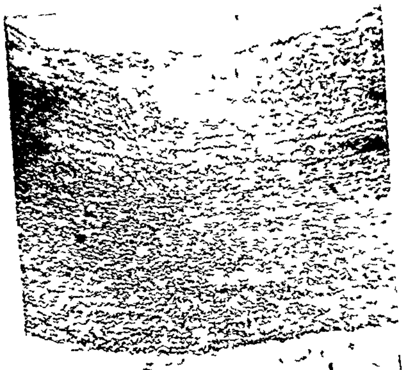


FIGURE 28c Aorta of 65-year old white male Verhoeff elastic tissue stain Note thickened intima and frayed elastic tissue x 50

ent in the cells and tissues in essentially the position they normally occupy One gets surprisingly good replicas of the cellular and tissue organization of the section This specimen is from a new born infant showing some slight measure of intimal thickening The elastic tissue runs as intact plates throughout the aorta Most of the adventitia has been stripped With microincineration (Figure 28b) we find essentially no mineralization of either the musculature or the elastic fibers that make up that young vessel A dark field preparation shows no mineralization of elastic fibers muscle or the intima in the young specimen

Figure 28c is a photomicrograph of the aorta of a 67 year old old individual in which elastic tissue has been stained by the Verhoeff method An adjacent section (Figure 28d) prepared by the microincineration technique reveals dense deposits of calcium ash which conform to the distribution of the elastic fibers This

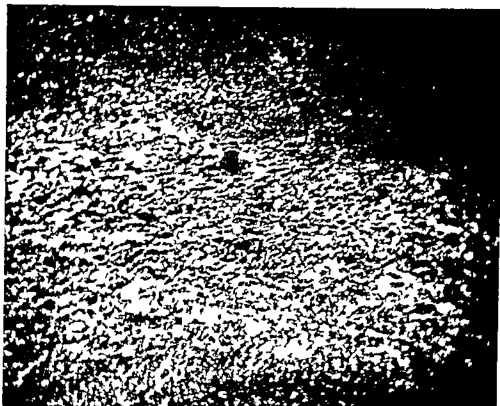


FIGURE 28d Same specimen as in No 28c Darkfield preparation of microincinerated section × 90

mineralization of the media is different from that we see in Moenckeberg's sclerosis While the latter represents a brittle concretion the former is a soft amorphous diffuse calcification which is at least to some extent organically bound It is revealed only by special procedures such as microincineration

Figures 29a and 29b show at a higher magnification the relation between the calcium ash and the elastic fibers This is a stripped media with the intima and adventitia both removed The section was stained with Verhoeff elastic tissue stain and we see again the plates of elastic tissue coursing throughout the media The microincineration shows the direct association of the mineral ash with the elastic tissue This of course lends itself only to semiquantitation In our next experiments we turned to direct quantitation

FIGURE 29a Media of human aorta with intima and adventitia removed Verhoeff stain x 280

FIGURE 29b Same as No 29a but incinerated x 280

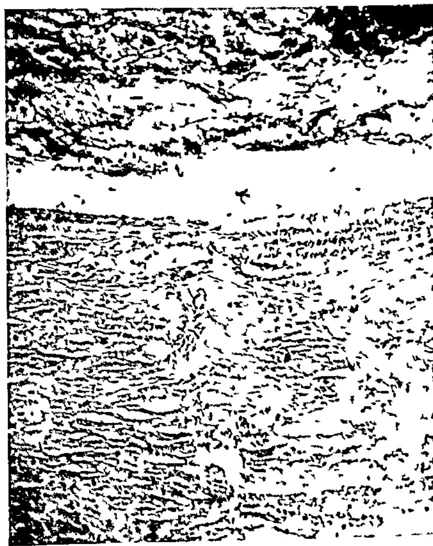


FIGURE 30a Aorta of 72 year old male Syphilitic aortitis H E x 100

Figure 30a is an aorta from an individual 72 years old with syphilitic aortitis with a loss of elastic tissue No mineral deposits are visible (Figure 30b)

To obtain quantitative data (Figure 31) on the age changes in elastic tissue we used a slight modification of the Lowry procedure for the preparation of elastin This uses 0 1 N sodium hydroxide extraction of the tissue at 98 C for 45 minutes Collagen muscle and various proteins are digested and hydrolyzed by the alkali and one gets after 40 minutes a very stable uniform preparation

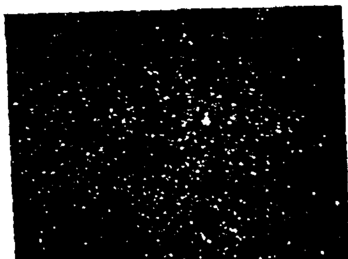


FIGURE 30b Same as No 30a but prepared by microincineration x 100

PER CENT INITIAL DRY WEIGHT

90 80 70 60 50 40 30 20 10

5 10 15 20 25 30 35 40 45 50 55 60

MINUTES

FIGURE 31 Graph illustrating hydrolysis of media of aorta in 0 1 N NaOH at 98 C A stable product (elastin) is obtained after extraction for 40 minutes

of elastin which is quite constant in its amino acid composition The data are concerned with the aorta from which both the intima and adventitia are stripped The analysis is of the elastin content of the media only The content of medial elastin as a function of age is represented in Figure 32 This graph is based on more than 100 analyses Relatively few cases less than 20 years of age are included I am not certain that the first two mean values on the left are statistically significant They may be slightly elevated because of the lack of adequate statistical data There is after 20 years of age a surprising constancy as a function of age in the content of elastin in the human aorta There is no loss of elastic tissue as such with age

Gutman Dr Lansing how did you determine the amount of elastin in your extract?

Lansing Direct weight analysis We can collect enough elastin from the aorta to get an accurate dry weight which is referred back to dry weight of the media There is no significant change in the amount of elastin in the aorta with age at least after the age of 20 If we take this same elastin which has been extracted from the media of the aorta and analyze it now for its calcium content we find again an exceedingly low calcium content prior to the age of 15 (Figure 33) After the age of 20 there is a gradual upsweep which is accelerated between 30 and 40 At 50 it is essentially maximal and tends to plateau at 6 percent calcium in elastic tissue through later years There is some measure of variation which probably depends to a large extent upon the region of the aorta from which elastin is drawn

Gofman What kind of people are these?

Lansing Most of the data here are derived from city hospital autopsies We have at least doubled this number with coroner's cases which are suicides automobile accidents murders and so on

Gofman These are not segregated on that basis?

Lansing No There are a number of variables which may account for the low calcium values as well as the high figures in Figure 33 It might be interesting to comment on these very high values actually some contain as much as 15 percent calcium a fantastic degree of calcification These are aortas from individuals with cirrhosis of the liver

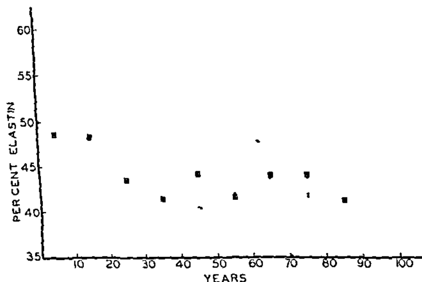


FIGURE 32 Graph showing the elastin content of the media of the aorta as a function of age There is no significant change with age

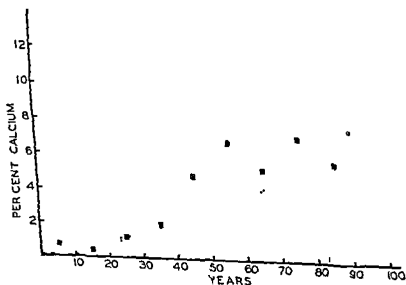


FIGURE 33 Analytical data for calcium content of elastin with age Calculated on basis of dry weight of elastin

Kendall Does this represent total calcium in the artery? Do you get any fractionation in your isolation procedure?

Lansing We can recover essentially all of the calcium since almost 95 96 percent of the arterial calcium is in the elastin

Simms Do you know whether it occurs as a calcium protein salt?

Lansing Unfortunately the answer is not simple If we take elastin extracted in alkali and suspend it in acid solution we can wash out all of the calcium In alkaline solution one can recombine approximately 15 to 20 percent of that calcium, which leads me to believe that only part of that calcium is organically bound to the elastin The balance may be in the form of something like the apatite crystal

Simms What about the phosphate?

Lansing The phosphate in elastin is practically zero in early life and it climbs with advancing years It approximates the ratio that one would expect in calcium phosphate

Kellner Is this apparent increase of calcium with age limited to the aorta or does it occur in other tissues such as the skin which has a good deal of elastic tissue?

Lansing We have done no quantitative analyses in vessels other than the aorta With microincineration and elastic tissue stains we find that the dermal elastic tissue with advancing age shows morphologic changes similar to those described in the arteries together with a profound increase in mineralization This is not unique As a matter of fact the elastic tissue network of the lung was described some years ago as undergoing fragmentation of the type that we associate with elacin and also mineralization

Gofman About eight or nine years ago S Hirsch did some studies of sections of various vessels including the aorta He stated that calcium deposited in association with elastin and that it was being missed in most pathologic specimens because of the use of acidifying medium

Lansing There is nothing novel about our own observations This is the first time that it has been quantitated

Grollman There are data to show for example that as an amoeba gets older, it, too manifests an increase in its calcium content The same is true apparently of protoplasm generally

Lansing My earlier studies on calcium changes with advancing age in various vertebrates and in invertebrates led me to a study of arteriosclerosis If we plot the age incidence of medical calcification we find the curve (Figure 34) rises sharply after the age of twenty with a plateau approached at 40 to 50 In this population of essentially six hundred specimens we find that the incidence of intimal thickening not distinguishing the type of thickening follows an irregular upward trend consistently trailing behind the incidence of medial calcification At least in this analytic series calcification in the media is progressing a decade before there is recognizable change in the intima on a purely morphologic basis Also intimal thickening of the aorta (20 29) becomes apparent at the age at which calcium levels of slightly over 1 percent are found in medial elastin This is the point that I want to make In all of the work we have done on the human aorta it seems that intimal

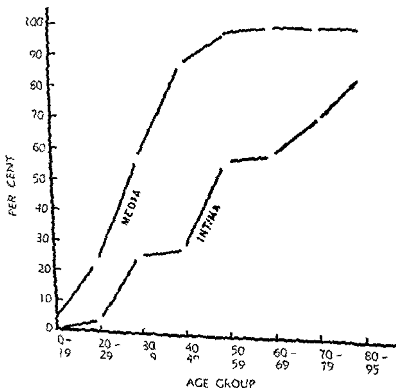


Figure 34 Percent incidence of medial calcification and intimal plaques as a function of age in approximately 600 human subjects Reprinted from article by Blumenthal, Lansing and Wheeler Am J Path 20 669 (1944)

thickening, with or without fat, is associated with a minimal concentration of at least 1 percent elastic tissue calcification of the media It represents a threshold level There is, however no direct correlation between the degree of intimal change and the degree of medial elastic tissue calcification *One can observe the same* grade of plaque with 6 percent or 1 2 percent calcium Medial elastic tissue calcification occurs with age in all individuals We have a few calcium values in old individuals that are low but again they hover around the 1 percent level There are no aortas that do not undergo some calcification with advancing age

Gutman Dr Lansing even if that is true for man, it would presumably not apply to other species in which the intimal thickening occurs at birth or develops later on

Lansing Patterson has indicated that medial age changes are *important in chickens*

Wilens What criteria do you use to determine whether or not the intima of the aorta is abnormally thickened?

Lansing Any elevation of the endothelium from the elastica with intervening fibroblasts connective tissue or muscle

Wilens At what age does this first occur?

Lansing It depends upon the vessel

Wilens When does it first occur in the aorta?

Lansing In the aorta it occurs I would say at about 10 years of age The first indications of elevation are in the 10 to 15 year age group

Katz In older people you do find aortas that are free of plaques? What are the calcium concentrations in such specimens? Do they have the greater percentages of calcium seen in older people? If so — and if you are right in relating this calcification to atherogenesis — how do you account for absence of atherosclerosis in these aortas?

Lansing I think the next chart (Figure 35) will help

Medial calcification *is not a consequence of the intimal change* since plaque free areas show medial calcification as a progressive function of age which may reach a level of 7 percent in the absence of gross intimal plaques

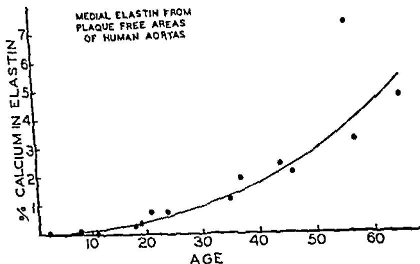


FIGURE 35 Calcium content of medial elastin as a function of age Samples were taken from plaque free regions of the human aorta

Katz My interpretation of that would be that therefore this medial calcification has nothing to do with the intimal lesions

Lansing The data indicates that medial elastic tissue calcification increases with age We have found repeatedly that this underlying medial calcification or some other medial damage is requisite to intimal change Since the medial calcification can occur without gross plaques it would seem that this is a necessary but not sufficient agent in the production of the intimal lesion

Gofman I am also interested in the question Dr Katz raised Suppose that you could get in the age group over 50 or 60 years a number of people whose aortas are relatively free of plaques would this curve be the same or different?

Lansing The aortas herein contained are referred to as normal aortas They show no gross plaques but I have as yet to see in essentially 1000 aortas which I have examined one which microscopically does not show intimal elevation I think we saw that in the photomicrograph of the young aorta that I projected a little while ago The intima is elevated It is necessary to qualify what we refer to as normal. There may be no gross plaque but microscopical examination shows that the intima is markedly elevated when compared to the juvenile

Katz Then essentially you say that all aortas develop this increasing calcification with age Then why consider that as a criterion as a necessary feature, for atherosclerosis if all aortas of this age will show it?

Lansing They show it in varying degrees

Katz They show it independently of whether they have atherosclerosis or not

Lansing You cannot have the intimal pattern without at least 1 percent calcification

Katz In the individual case?

Lansing In the individual case

Stamler When you speak of the intimal pattern do you mean merely this pattern of thickening? Intimal fibrosis and atherosclerosis are not the same entities

Lansing I am deliberately not describing it either as the fibrosed or the fatty or necrotic lesion With any elevation of the intima there must be at least 1 percent calcification of the underlying elastic tissue in the aorta

Gutman Have you had opportunity to examine the aorta of a young person with let us say nephrosis who had marked intimal plaque formation — atherosclerosis? Is calcification of the media found at that age?

Lansing Yes in the younger age group between 30 and 39 there is four to five times as much calcium as in a corresponding normal group

Barr Does that include any xanthoma?

Lansing I am sorry I cannot say

I have described the pattern of elastic tissue aging for the aorta on both a quantitative and qualitative basis There is a critical level of 1 percent calcification required to get the intimal elevation This is diffuse calcification it is not the Moenckeberg type Comparable age changes occur in the muscular arteries such as the coronary hepatic and renal



Figure 36 Renal artery of 19-year old male Resorcin fuchsin stain Note beginning penetration of elastic elements into media x 90 Lower figure microincinerated section of same specimen x 90

Figure 36 typifies what we have found The muscular vessels show essentially the same general elastic tissue change as the aorta This is the renal artery of a young individual stained by the Verhoeff method The elastica is intact with no extensions from the internal elastica into the media The endothelium rests directly upon the elastica There are several bands of elastic tissue in the external lamella which is quite characteristic of the renal artery Even at this age (19 years) there are extensions of elastic tissue from the external elastic plates up into the media This is the earliest type of change In still younger specimens there are only two or three elastic plates in the external lamella and there are no such extensions in the media I am well aware that the classical textbooks of histology do describe this as a normal situation for the coronary artery the aberrant elastic material is shown coming down from the other direction Each vessel has its own detailed pattern of change and the age at which these changes begin varies

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Katz They *show it independently of* whether they have atherosclerosis or not

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FIGURE 37 Renal artery of 62 year-old male Above Resorcin fuchsin stain showing elastic fiber extensions into media x 90 Below microincinerated section There is pronounced calcification of the elastic tissue x 90

Barr Have you encountered any cases in which there was hyper calcemia due either to vitamin D or to hyperparathyroidism?

Lansing Not in this group at all We tried parathormone vitamin D elevated calcium adrenalin etc in the mouse in an attempt to produce these age changes with no apparent effect

Katz Several workers have shown in both man and animals that excessive intake of vitamin D results in calcification and rigidity of blood vessels

Barr One may see in vitamin D intoxication and in rare cases of hyperparathyroidism calcification of the media of vessels which really can be classified as Moenckeberg's sclerosis

again with the vessel In the coronary, thickening of the intima occurs at an early age with extensions of elastic tissue reaching down from the internal elastica but practically no change in the external The hepatic vessels develop elastic tissue fraying from both the external and internal elasticas fairly slowly somewhere between 20 and 30 years of age The renal differs from both the coronary and hepatic in that the fraying extends from the external lamella only Here in Figure 36 are shown the early stages of aging of the renal artery In the microincineration the internal elastic lamella shows a slight degree of calcification There is little calcium in the media and only small amounts in the external lamellae

In the next photograph (Figure 37) we have a typical old renal artery There are thin shreds of elastic tissue in great amounts throughout the media The inner elastica is still intact There is no appreciable thickening of the intima In a few spots we could demonstrate some connective tissue The external lamellae have duplicated and reduplicated In the microincinerated preparation there is a dense solid band of calcium associated with the external lamella These are not elastic fibers nor isolated fibrils They are plates of elastic tissue that literally make a collar about the vessel In youth the collar is very elastic but with age it becomes heavily calcified This is in effect a cuff around the renal vessel — a calcified plate — which may bear upon some of the physiologic changes in the renal artery

In Moenckebergs sclerosis some of the vessels studied show the preceding but a more pronounced pattern exists There are *dense nests of calcium which invariably correspond to massive* accumulations of aberrant elastic tissue (elacin) Each specimen *with Moenckbergs sclerosis studied shows dense nests of elastic* tissue and of course the mineral I suspect that Moenckebergs *sclerosis is simply a version of the elastic tissue aging that occurs* in a coronary renal or hepatic artery It is again the elastic tissue *that changes with age and calcifies In Moenckebergs sclerosis* the senile type of elastic tissue happens to aggregate into a hard mass *while in these other vessels it remains diffuse* I see no difference in principle between the elastic tissue aging of elastic or muscular *vessels including Moenckebergs sclerosis*

Wilens Have you ever seen Moenckebergs sclerosis of the aorta?

Lansing There are a few in the colored population that Blache and Handler have been working with In our white series — which numbers close to a thousand now — I have seen none

Plaques are not the same at all ages The grossly normal intima reveals small but significant increases in both calcium and cholesterol the fatty plaques show no age changes but necrotic plaques in the older age group contain much more calcium than comparable plaques of the younger group

The fibrous plaque in the age group 30 to 59 has 0 6 percent calcium at 60 to 80 1 6 percent The significant thing here is that the cholesterol content in the old group is much higher than in the young group and approximates closely the cholesterol content of the fatty plaque at either age There is the possibility or the implication that the fibrous plaque is progressively becoming more like the fatty plaque both in regard to its mineral content and cholesterol content

Simms By fibrous you mean there was no stainable fat?

Lansing No stainable fat The necrotic plaque also changes with age Its calcium doubles essentially which again suggests that the fat is becoming the *necrotic* type The mineral content goes up markedly and because the mineral is going up the cholesterol has to go down on a purely arithmetic basis

Katz Isn t it possible — just again taking the opposite point of view — that if atherogenesis is an episodic process each deposit of cholesterol leads to some calcium deposit? May not fibrosis and calcification be secondary phenomenon occurring latterly in the course of transformation of an atheromatous plaque into an atherosclerotic plaque?

Lansing I find it difficult to reconcile your view with the data If it is regression one should find a progressive increase of the fibrous plaques with age not necessarily but it would be a reasonable expectation

Katz Calcium does not disappear Once it is deposited it is hard to get rid of Cholesterol can be deposited and removed

Gofman Each time cholesterol is deposited there *is an* increase in the fibrous plaques so that the older fibrous plaque would have a higher cholesterol content than the earlier fibrous plaques and would in no way *afford* evidence that the fibrous deposit precedes the cholesterol deposit

Dock. These changes occur later or not at all in the normal pulmonary artery.

Lansing. Arteriosclerotic changes are not very prominent in the pulmonary circulation and the data show that the calcific changes in the media are also retarded.

We duplicated to some extent the experiments done by Weinhouse and Hirsch(2) and several other workers in isolating plaques differing essentially in only one important and I think critical step. We separated the intima from the media and analyzed those separately. The adventitias were always discarded in our analyses. We have now isolated the intima from the media and classified the intimas on the basis of grossly normal (no perceptible lesion) the glistening white flat fibrous plaque, the gross fatty plaque and finally the ulcerating and calcified plaque. These are arranged to bear the implication of seriation that may or may not be justified. The data support the view that the fibrous plaque is the youngest of the intimal lesions (Table XI).

TABLE XI

	Percent Calcium		Percent Cholesterol	
	Age 30-59	Age 60-80	Age 30-59	Age 60-80
Normal	0.17	1.54	1.9	3.2
Fibrous	0.61	1.62	8.5	24.8
Fatty	6.62	6.17	29.9	29.3
Necrotic	5.56	10.90	47.8	24.5

Analytical data for calcium and cholesterol contents of isolated intimal plaques in two different age groups. Grossly comparable plaques may differ markedly depending upon age of the individual.

In these groups of plaques we analyzed the calcium and the cholesterol contents of the plaques. One series of plaques was collected from individuals 30-59 years of age and the second group from individuals 60 to 80 years of age. The age factor has not been considered in earlier chemical analyses. This is an important point since it is apparent that grossly comparable plaques may vary strikingly in their chemical composition.

The grade 1 the fibrous type in the age group of 16 to 24 years of age has an incidence of 68 percent at 25 to 29 years 66 percent 30 to 34 years 27 percent and so it goes — 40 to 49 years 0 7 percent

The grade 2, the fatty type begins in the 18 to 24 year age group at 20 percent incidence a low level and progresses with age to 18 percent It shows a transient peak at 30 to 34 and levels off at 24 percent The calcified type the fatty necrotic type begins at 12 percent the lowest frequency is in the youngest group 24 and moves up steadily to 75 percent at 40 to 49 years

Wilens Calcified intima plaques are generally considered to be hyalinized plaques that have secondarily become calcified As this occurs the number of hyalinized non calcified plaques may become reduced in number I believe it is possible in this fashion to account for the diminishing number of hyalinized plaques shown in Yater s data on types of coronary artery plaques

Lansing His lesion involves nests of cholesterol crystals with granules of calcium therein He is describing the fatty plaque with calcium We are not talking of the flat calcareous plate that contains fibrous connective tissue and calcium which of course we have all seen I think that is a different situation entirely

Cofman Lipid infiltration is the basis of plaque formation If many of the scattered statements and observations in the literature such as Leary s are correct that there is increasing difficulty of removal of lipids with increasing age the whole picture would be just as consistent on the basis of lipid infiltration In a youthful plaque there is a greater ease of removal so you see a higher percentage of fibrous plaques with increasing age there is a greater difficulty of lipid removal and you will see a lower and lower proportion of fibrous plaques which would account for Yater s data on just the opposite hypothesis

Lansing Again we are getting back to whether it is essential to have the fibrous preceding the fatty plaque I do not believe it is essential but probably is the usual sequence in plaque development As far as I am concerned this manner of seriation of plaques is reasonable

It gains merit in the light of the next experiment (Figure 39) We have again isolated plaques but in a single age group (30-39 years) rather than different ages We have isolated plaques by

Lansing Then you should find an increase in fibrous plaques with age That in the human is entirely unwarranted on the basis of direct observation

Stamler Dr Lansing that would not be so if fibrous plaques are focal sites of predilection for secondary re deposition of lipid with superimposition of fresh atheromas on old fibrous plaques

Lansing I think we can argue away from the descriptive evidence I am sure there is always an alternative possibility, but at least this is a reasonable interpretation of the data

Our interpretation appears to be a reasonable one particularly when we take Yater s(3) figures (Figure 38) into consideration In the coronary – I have been talking about the aorta – in the coronary he uses a grading of plaques that is very comparable to ours

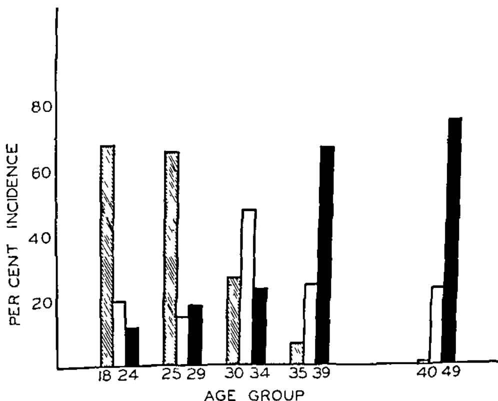


FIGURE 38 Incidence of various grades of plaques in the human male coronary artery at different ages Striped bars represent the fibrous type plaque solid white represent the fatty plaques and solid black represents the severe ulcerated plaques Calculated from data of Yater

Katz Your assumption is that the medial calcification precedes intimal plaque formation

Lansing If this seriation is right

Katz Assuming this seriation is right isn t it possible that intimal plaque formation may be the cause of calcium deposition in underlying medial elastin so that calcium in medial elastin may be a secondary result of an intimal lesion rather than the reverse?

Lansing Except that we have already seen that the medial elastic tissue change can occur without intimal change

Katz That does not rule out this other possibility I agree to the fact that it has occurred but it is not an answer to my question The question is may not the reverse sequence occur?

Lansing If we did not have these analyses I would say yes, it can be reversed I believe that medial elastic material calcification either in muscular arteries or the aorta is requisite to intimal change but not a sufficiency Other factors operate with the alteration of the arterial wall to produce plaques What these factors are I am not prepared to say Diffusion mechanisms vasa vasorum or compensatory reactions may be involved

Stamler May I ask why you chose to arrange the sequence in an ascending order of cholesterol concentration rather than an ascending order of calcification? If you arrange it in an ascending order of calcification you could just as readily infer the other sequence that the cholesterol is initially higher it is removed in the chronic process in the chronic process fibrosis proceeds progressively and then calcification proceeds progressively I do not want to deny that both are possible I want to make the point that this is not a controlled experiment These are observations on pathological material

Lansing These are descriptive data only I started out by saying, the whole experiment depends upon the validity of the seriation The arrangement here is a function of this seriation and not a function of the calcium level or the cholesterol I do not believe it would be proper to adjust the rationale of this experiment after the data are obtained

We will go now to the pulmonary artery which I think is important in these discussions Like the aorta it has its origin close to the heart and is also an elastic artery If the calcification of

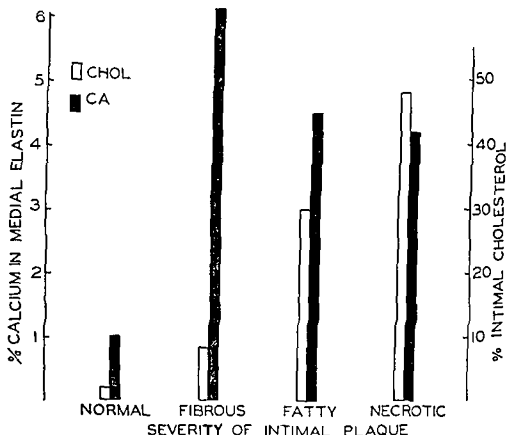


FIGURE 39 Histogram of calcium and cholesterol analyses in a series of plaques graded by severity Calcium is expressed as percentage in dry fat free medial elastin cholesterol as percentage in dry intima

punching separated intima from media discarded the adventitia, and analyzed the calcium content of the media and the cholesterol content of the overlying intima

In this restricted age group we collected normal intimas fibrous fatty and necrotic plaques The calcium content of medial elastic tissue is maximal in the transition from normal to fibrous and if anything drops off thereafter The cholesterol content of the plaques is low in the normal intima and fibrous plaques which of course we would expect from general observations It increases in the transition from fibrous to fatty to necrotic

Katz May I get some interpretations? The cholesterol is in the plaque intima overlying the elastic media that you are analyzing?

Lansing Yes

Figure 42 gives quantitative data to go along with the histochemical and histologic data on a series of pulmonary arteries compared with the aorta The elastin content does not change with

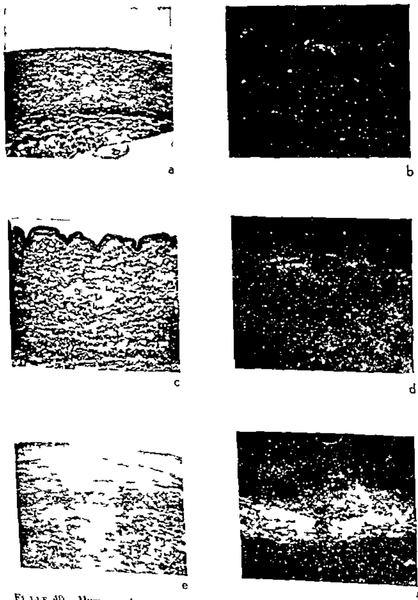


FIGURE 40 Human pulmonary arteries at different ages with and without intimal plaques

medial elastic tissue as demonstrated in the aorta is requisite to intimal plaque formation then the senile pulmonary artery should be relatively free of this elastic tissue age change.

Figure 40 illustrates several pulmonary arteries at various ages stained for elastic tissue and also microincinerated. The infantile pattern is shown in a & b with the elastic tissue immediately under the endothelium. There is actually no mineral in the elastic elements. Next (c & d) is a senile pulmonary artery with little or no change in the elastic lamellae, no hyperplasia or thickening of *the intima and no calcification of the elastic elements of the media.* This obtains in 99 out of 100 older pulmonary arteries.

In cases of increased arterial pressure, such as in mitral stenosis, atheroma do occur, and in such cases, as shown in e & f, there is significant breakdown and calcification of the medial elastic tissue. Here the plaque has some fine threads of elastic tissue running through it. The elastic *tissue is broken up.* The elastic lamellae that make up the wall of the vessel are again fractured and aggregated and this vessel now resembles the aorta. Upon analysis it shows more than 1 percent calcification.

Figure 41a is representative of the 12 to 15 mitral stenoses we have studied. Plaques are evident, there is utter destruction of the elastica and of the elastic plates with the presence of this peculiar aberrant elastic tissue which has an affinity for calcium. It is granular, it stains deeply, it is irregular in shape. Next to it (Figure 41b) is the microincineration which again demonstrates the accumulation of calcium. Elastic tissue calcification ordinarily not seen in the pulmonary is apparently present in mitral stenosis. This case involved 3 percent calcification.

FIGURE 40 a Pulmonary artery of a 21 year old colored female. Verhoeff elastic stain x 50

b Microincinerated section of specimen shown in Figure 40a. Darkfield x 50

c Pulmonary artery of a 67 year old white female. Resorcin fuchsin x 50

d Microincinerated section of specimen shown in Figure 40c. Darkfield x 50

e Pulmonary artery of a 69 year old male with intimal plaques. Verhoeff elastic stain x 50

f Microincinerated section of specimen shown in Figure 40e. Darkfield x 50

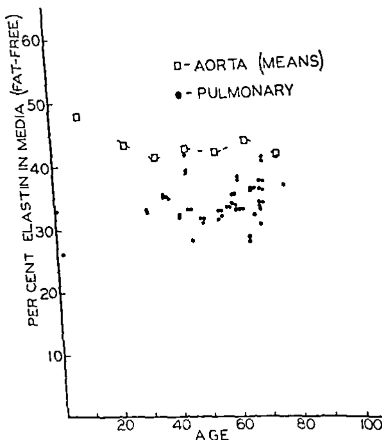


FIGURE 42 Changes with age in elastic tissue of human pulmonary arteries compared with those of the aorta

age There is less elastic tissue in the pulmonary than in the aorta and it stays remarkably constant as a function of age

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FIGURE 41 a Pulmonary artery of a 47 year old white male with history of mitral stenosis A thick intimal plaque is present elastic tissue is frayed and fragmented. Verhoeff x 100

b Same specimen as No 40a Microincinerated Note intense calcification of the media x 100

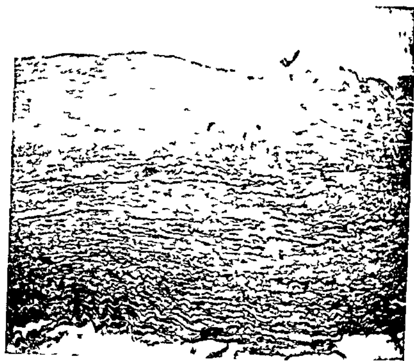




FIGURE 41

artery are very much retarded and when they are present there are also atheroma present The latter are apparently entirely a function of the pressure within the pulmonary artery

Table XII illustrates the factor of disease in the incidence of elastic tissue calcification It is a summation of roughly six hundred cases graded according to severity of calcification age sex and disease The nephroscleroties and the hypertensives are significant only in the young age groups The severity index in hypertension is 2 8 in the decade 30 to 39 The normals in that decade are 0 6 and 0 8 for males and females respectively Syphilitic aortitis shows no calcification of elastic tissue There is no elastic tissue or essentially none in the luetic vessel However plaques are prominent

The coronary thromboses do not deviate significantly from the total averages of both sexes Data for individuals younger than 40 are lacking so no conclusion can be reached as to the situation in early life

Stamler Would you care to comment on the superimposition of atherosclerotic plaques on the fibrotic lesions of syphilitic aortitis in the absence of calcification of the media?

Lansing All I can say is first calcification does not occur *secondly* if my premise is sound that medical damage is requisite to plaque formation medial scarification of luetica aortitis would constitute another type of medial damage The usual medial change that we find with the passage of time in the human is the elastic tissue change but it is not the only one the luetic pattern is *another means* of disruption for the vasa vasorum But the most common medial damage is the elastic tissue change of aging

Katz Is your argument that medial pathology is primary based on the assumption that interference with the vasa vasorum is a key change?

Lansing No I am offering the vasa vasorum as a possible suggestion Last November Woerner at Louisville demonstrated some of his injected preparations of the dog aorta and the vasa vasorum thereof The vasa vasorum of the dog aorta is restricted to the outer third of the media the inner two thirds contain none of the small arterioles From his preparations one cannot determine how far the capillaries penetrate The pattern in his injected specimens is such that if cross matched with some of my slides the avascular region in his preparations corresponded to that which showed

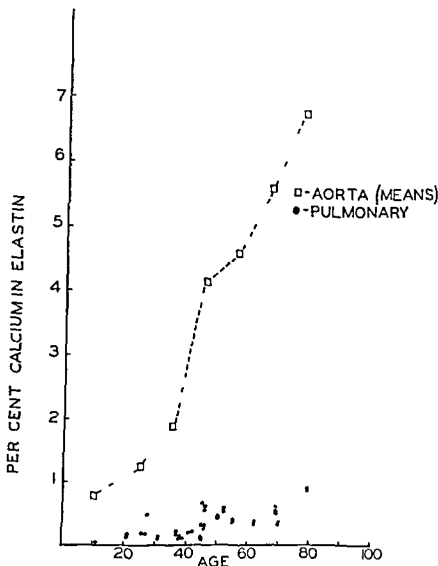


FIGURE 43 Increase of calcium content of pulmonary elastin with age compared with similar analyses of aortas

Figure 43 is a comparison of the calcium content of the elastic tissue of the pulmonary artery with that of the aorta The pulmonary is not entirely free of the calcific age change which although retarded in this vessel shows a tendency to rise with the passage of time All the calcium values are lower than 1 percent except four and each of these four is a mitral stenosis This value of 3 percent is from the same artery which was sectioned and illustrated in Figure 41a The medial elastic tissue changes in the pulmonary

elastic tissue changes early So it is possible that vasa vasorum are concerned in arteriosclerosis

Gofman Is there agreement among pathologists that athero sclerosis is less severe in the young female as compared with the young male below the age of 40?

Wilens It depends on whether you are referring to atherosclerosis with plaque formation or simple atherosis meaning by that the formation of lipid streaks or patches in the intima Simple lipid streaks such as Aschoff(4) used to refer to as atherosis as distinct from atherosclerosis of older age groups are equally common in young persons of both sexes

Kellner Clinically significant atherosclerosis in terms of coronary sclerosis is probably more common in the young male than in the young female certainly if the female does not have diabetes or hypertension

Gofman Leaving out diabetes and hypertension do you feel that the amount of overall aortic atheromatosis is about the same in the two sexes?

Wilens The simple lipid streaks or diffuse lipid deposits which may or may not lead to plaque formation are found with equal frequency in both sexes in the intima of the aorta

Gofman What about plaque formation?

Wilens Plaque formation is infrequent in both sexes before the age of 35 but tends to be slightly more common in males

Kendall I find that I am very much confused by the method in which you present some of these data That is where you showed the correlation between calcification of the media and degree of percentage of cholesterol in the overlying plaques the calcification was expressed as percent that is calcium as percent of elastin You have two variables there You are comparing a ratio between two independent variables with a direct quantity That is you are comparing a ratio with a quantity in that case What is the correlation between total calcium in the underlying media and the total cholesterol? By the chemical methods that you are using for isolating elastin the total calcium in the aorta would be expected to remain in your preparation whether or not the total amount is associated with the elastin However your microincineration does indicate that the calcium is associated with the elastin

TABLE XII

The Influence of Sex and Disease on the Graded Incidence of Deposition of Calcium in the Media of the Aorta as Correlated with Age

Classification	Age Groups							
	0 19	20 29	30 39	40 49	50 59	60 69	70 79	Over 80
Females (all cases)	00±00	04±02	08±02	12±01	17±03	16±01	17±01	18±02
Males (all cases)	01±01	01±01	06±02	12±01	15±01	17±01	18±01	21±02
Accident or acute disease	00±00			10±02	12±01	16±01	20±02	19±03
Tuberculosis	01±01	03±02	06±01	12±03	14±02	16±02	19±02	
Cancer			10±06	11±02	18±01	18±01	13±01	23±03
Nephrosclerosis and/or hypertension			28(4)	16±02	18±03	15±01	15±02	18±03
Coronary thrombosis cerebral thrombosis and hemorrhage				13(4)	16±02	17±02	19±02	21±04
Syphilitic aortitis			00(3)	00±00	02±01	04±02	01±01	——

Figures in parentheses represent the number of cases when they were relatively few
Reprinted from article by Blumenthal Lansing and Wheeler *Am J Path* 20 671 (1944)

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3 YATER W M *et al* Coronary artery diseases in men 18 to 39 years of age report of 866 cases 450 with necropsy examinations *Am Heart J* 36 334 (1948)

4 ASCHOFF L Introduction pp 1 18 in Cowdry E V *Arteriosclerosis A Survey of the Problem* New York Macmillan 1933

Lansing And also — perhaps I did not make it clear before — if we analyze aliquots of fresh media and of elastin for total calcium we find that at least 95 percent of the calcium is in the elastin We analyzed the elastin content of the media underlying the plaque and the calcium content of that elastin There is no significant change in the elastin content, with minor variations except in the most severe plaques where we have very gross changes There is with the compression of the media some loss of the elastic tissue But even in such cases the calcium is still high

Kendall I thought it was interesting that in your older age groups the fibrous plaques contained as much cholesterol as did those that appeared to be essentially deposits of lipids Of course, Schoenheimer reported years ago that aortas in which the lesion seems to be entirely fibrotic in which no lipid at all can be demonstrated by histologic stains frequently contain much more cholesterol than lesions which grossly appear to be essentially fatty

Lansing You can see from the data I showed that if one does not consider the age of the individual from which a plaque is taken one can get remarkably different values This may be a source of confusion in Weinhouse's paper (2)

I will briefly summarize Arteriosclerosis appears to be a dual process involving aging of the arterial wall and cholesterol accumulation in the intima Calcification of senescent elastic tissue in the media occurs with or without atheromatosis When the two co exist analyses indicate that cholesterol accumulates in the intimal plaque after the underlying media has undergone elastic tissue calcification What factors condition the age change in chemical composition of elastic tissue remain to be determined Nothing in these data should be interpreted to mean that altered cholesterol metabolism is not a factor in arteriosclerosis We wish only to stress that aging of the arterial wall establishes a substrate for penetration and accumulation of cholesterol It is possible that without such changes in the arterial wall cholesterol will not accumulate in the lining of arteries

These views are based entirely upon descriptive analyses of human arteries Experiments are needed to confirm our interpretation of the descriptive evidence We are merely insisting and will continue to insist that there are changes morphological and chemical in the media which as far as we are concerned must be considered in discussion of the genesis of arteriosclerosis

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STUDIES ON EXPERIMENTAL ATHEROSCLEROSIS AND HYPERTENSION

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HYPERTENSION WILL be a minor part of this presentation. I am here as spokesman for our department's atherosclerosis research group which over the years has included the late Doctor Deborah V. Dauber, Doctors Louis Horlick, Jeremiah Stamler, and Simon Rodbard, plus the technical staff. We will talk about atherosclerosis and not arteriosclerosis, about the intimal lesion which is initially a lipid laden foam cell cushion (so called "pure" atheroma) and which in the course of its evolution undergoes sclerotic changes. We have concluded that this is a pathologic process distinct from (although often accompanying) aging; that it is not inevitable; that these two aging-inevitability dogmas (and they are dogmas which die hard) have done much to prevent productive work in the field of atherosclerosis.

We adhere to the cholesterol concept of atherogenesis, i.e., that atherosclerosis is a disease of altered lipid metabolism and that the offending agent is cholesterol, whether it be cholesterol *per se* or its state of plasma and tissue lipoprotein colloidal aggregation and the related alterations in cholesterol transport, ingestion, excretion and metabolism which this state reflects. This general cholesterol concept of atherogenesis we regard as the essential frame of reference within which it becomes possible to carry through an intensive, ultimately successful research assault upon the specific aspects of the overall problem.

I began a study of coronary disease at the Michael Reese Hospital about 21 years ago. After a decade it was abundantly apparent that the basic problem in coronary disease was atherosclerosis. At that time, the animal used by most people was the rabbit. Also at that time a considerable weight of opinion prevailed that since cholesterol was a foreign dietary substance to the herbivorous rabbit, observations in the rabbit were not germane to man. Dr. Deborah Dauber and I undertook to find an experimental animal that would be less subject to such criticism. The work of Fox on

birds and some Japanese work on chickens gave us the idea that we should study the fowl The chicken is omnivorous like man Dr Dauber found that it has spontaneous gross lesions of the aorta that in their fully developed form are morphologically atherosclerosis We were readily able to demonstrate that cholesterol feeding induced atherosclerotic lesions that were distinct from the spontaneous and that simulated even more closely the lesions of man Chicks proved to be readily utilizable for extensive chronic experimental studies

Over the years through trial and error we developed a well integrated research team with considerable "know how" in this field We wish to stress the need for proper control of all studies Control groups of adequate size are essential with every experiment Data on feed intake and rate of weight gain are imperative Adequate methods of biochemical and pathologic quantitation (gross and microscopic) with adequate insurance against personal preconceived notions are also essential With respect to this latter problem we developed a technic of sacrifice whereby two observers grade vessels for lesions with neither of them knowing anything about the animal other than its code number In this manner specimens from several groups indiscriminately mixed are serially examined and graded We routinely examine grossly the thoracic and abdominal aorta and the brachiocephalic and iliac vessels and grade them from 0 to 4 on a purely arbitrary scale

As already indicated our early work made it clear that the chick was suitable for the study of two types of lesions the spontaneous and the cholesterol induced The spontaneous lesions are primarily in the abdominal aorta In animals originally studied obtained from the slaughterhouse considerable quantities of lipid were present in the fully developed spontaneous plaques However in birds reared from hatching on the carefully controlled diets of our laboratory we do not see as much lipid histologically as we saw in the original birds This finding has led us to re affirm the suggestion of Dauber that the spontaneous lesion may be initially a fibrotic process with lipid deposition occurring (if at all) only secondarily Further work on this problem is proceeding

In cholesterol fed chicks the initial lesion is definitely intimal atheroma Sclerotic changes apparently occur secondarily they include lipophage disintegration fibroblastic accumulation of lipid deposition of cholesterol crystals formation of lipid abscesses fibrosis compression and invasion of the media calcification cartil

age and bone formation Thus we see the entire spectrum of changes observed in human lesions excepting ulceration and thrombosis These cholesterol induced lesions occur first in the thoracic aorta in contradistinction to the spontaneous type With either high percentages or prolongation of cholesterol feeding the entire aorta tree is involved and considerable lipid deposition and atherogenesis proceed in apparently pre existent spontaneous fibrotic plaques of the adominal aorta A few years ago a third type of experimental *lesion was produced in the chick and became available for study* Lindsay Chaikoff *et al* showed that chronic administration of stilbesterol results in prolonged hypercholestermic hyperlipemia in cockerels Atherosclerosis eventually supervenes particularly in the thoracic aorta The lesions are atheromatotic and atherosclerotic in type The possibility of studying 3 different types of lesions in chicks spontaneous cholesterol induced and stilbesterol induced makes this animal particularly useful for research in this field We have carried out considerable work on all 3 lesions

Following our preliminary studies one of the first things under taken was the quantitation of the interrelationship among per centage of dietary cholesterol duration of cholesterol feeding degree of hypercholesteremia and intensity of atherogenesis In the original study of this type by Horlick and myself and in suc ceeding experiments quantitative interrelations were demonstrable as illustrated in Tables XIII XIV and Figure 44(1) Within limits both degree of hypercholesteremia and severity of atherosclerosis increase as the percent of cholesterol in the diet is raised and/or the duration of cholesterol feeding is increased The limits are with diets containing greater than 2 percent cholesterol no further increments in hypercholesteremia and atherosclerosis occur simi larly if cholesterol feeding be prolonged maximal severity of gross *atherosclerosis (grade 4) eventually supervenes with the higher* percentages of dietary cholesterol Time does not permit a detailed exposition of the auxiliary problem of the influence of dietary neutral fat on cholesterol induced hypercholesteremia and athero genesis Suffice it to state that when cholesterol is added to com mercial chick starter mash (normal fat content 3 5 percent) hyper cholesteremia and atherosclerosis develop without the addition of any cottonseed oil to the mash

In all birds receiving the higher percentages of dietary cholesterol the plasma is grossly hyperlipemic increments *of cholesteremia* are several fold (Table XIII) To clarify the problem as it pertains

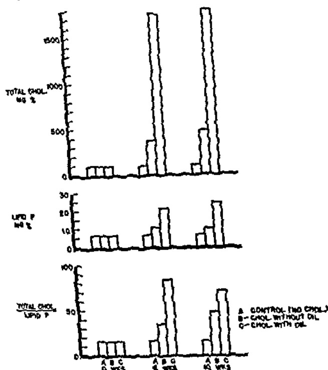


FIGURE 44 Lipemia in chicks fed ¼% cholesterol diets with and without 20% cottonseed oil Reprinted from Katz and Stamler *Experimental Atherosclerosis* Charles C Thomas Springfield Ill *in press*

to man it appeared desirable to work with levels of plasma cholesterol of the order that occur in man Stamler and our group accordingly studied the effects of ¼ percent cholesterol diets since these produce a minimal hypercholesteremia and organ cholesterosis comparable to the degrees of hypercholesteremia frequently seen in man with coronary atherosclerosis Some data on plasma cholesterol levels and atherosclerosis with this ¼ percent cholesterol diet are presented in Table XIII and Figure 44 Two different experiments of this type are included In the first when the cholesterol diet was begun in 5 week old cockerels and continued for 35 weeks the mean plasma total cholesterol level was 166 mg percent (control 99) No hyperphospholipemia prevailed thus the C/P ratio* was elevated In series 2 placed on diet on the day after hatching and continued for 15 weeks mean plasma total cholesterol was 140 mg percent (control 90) In both series gross atherosclerosis of the cholesterol induced type was found in the thoracic aorta (the con

*plasma total cholesterol / lipid phosphorus ratio

TABLE XIII*

Effects of Various Concentrations of Dietary Cholesterol on Cholesteremia, Using Diets Containing 0 to 5 percent Cottonseed Oil†

Series 1		Series 2	
Diet	Plasma Total Cholesterol mg %	Diet	Plasma Total Cholesterol mg %
Plain Mash	78	Plain Mash	92
¼ C O	150	¼ C O	109
½ C O	270	½ C O	242
2 C	467	2 C	365
2 C O	638	2 C O	505

† All groups had similar rates of feed intake and weight gain Series 1—chicks placed on diet at 5 weeks of age Series 2—at 1 day of age Bleedings in both series at 10 weeks of age ¼ C O = ¼% cholesterol + 5% oil added to mash ½ C O = ½ cholesterol + 5% oil added to mash 2 C = 2% cholesterol added to mash 2 C O = 2 cholesterol + 5 oil added to mash

TABLE XIV*

Effects of Various Concentrations of Dietary Cholesterol on Incidence and Severity of Lesions, Using Diets Containing 0 to 5 percent Cottonseed Oil‡

Diet	% Birds with Lesions	% Birds with Lesions Grade 1 or >	Mean Grading of Birds with Lesions	Mean Grading for all Birds in Group
Plain Mash	0	0	0	0
¼ C O	0	0	0	0
½ C O	50	50	1 5	0 7
2 C	80	80	2 0	1 6
2 C O	91	73	1 7	1 5

‡ 20 weeks of age 15 weeks on experimental diet For plasma cholesterol levels see Table XIII For symbols see Table XIII

* Reprinted from Katz and Stamler *Experimental Atherosclerosis* Charles C Thomas Springfield Ill in press

trol had no such lesion) In these particular experiments analysis of lipid data on individual birds revealed no correlation between degree of elevation of C/P ratios and severity of aorta athero sclerosis In contrast degrees of hypercholesteremia and athero-sclerosis did correlate

Thus in birds with plasma cholesterol levels of 175 mg percent or greater 100 percent had lesions whereas in those below 175 mg percent none of them did From this experiment we came to the conclusion that with respect to pattern of cholesteremia we have imitated in the chicken the situation frequently occurring in man namely atherogenesis proceeding with but slight elevations of plasma cholesterol We are now sending samples of serum from chicks fed this ¼ percent cholesterol mash to Dr Irvine Page for ultracentrifuge analysis of S_f patterns compared with birds on a regular diet

Gofman With cholesterol levels of 125 to 150 mg percent in the chicken you already have abnormal lipoprotein molecules?

Kellner Your figure of ¼ percent cholesterol in the diet is difficult for me to interpret What does it mean in terms of mg of choles terol per kg of chicken so that we may compare it with the choles terol intake of a dog or rabbit?

Stamler 0 25 grams of cholesterol mixed with 5 00 grams cotton seed oil + 94 75 grams of mash The feed intake of all but baby chicks is roughly 100 grams a day The mature weight of these cockerels is 2000 to 2500 grams such birds take in about 100 *grams* of feed a day

Kellner That would be 250 mg of cholesterol per kg which is considerably larger than the dose required to produce athero sclerosis in rabbits I do not know how that compares with Dr Kendall's dose in dogs on a weight basis

Katz Such comparisons are difficult to make As I recall most rabbit experiments involve ingestion of 0 5 1 0 gm of cholesterol daily a greater amount than we used in this experiment Metabolic differences between avian and mammalian species must be taken into account The chick has a much higher body temperature and metabolic rate than man or rabbit An intake of 100 gm of this diet yields well over 400 calories to the chick On a per kg proportional basis man would have to ingest 14 000 calories per day and 100 gm of cholesterol Dr Stamler has done considerable

calculating of this sort with respect to species comparisons Their validity is difficult to substantiate We make only one assertion that atherogenesis proceeds in cholesterol fed chicks with levels of cholesteremia similar to those usually seen in atherosclerotic man This is not an insignificant finding since one of the old objections to experimental cholesterol atherosclerosis was that it was associated with a degree of hypercholesteremia and organ cholesterosis such as to render it not at all comparable to the situation in man (excepting xanthomatosis)

Kellner It is certainly possible to produce atherosclerosis in rabbits whose sera are not milky and whose cholesterol levels are on the order of 200 mg percent

Katz That I know—Anitschkow proved it many years ago I would like to know about the dog

Gofman Milkiness of serum is only an indication of light scattering Light scattering can be due to molecules that are down around the size of a serum albumin molecule or it can be due to molecules that are a million or fifty million times as large The effect depends on how many there are The S_f 10 to 20 group for example can be present at appreciable concentration but the serum will not be milky On the other hand milkiness alone is not a criterion that atherosclerosis will appear because some of Dr Kellner s animals receiving detergents had very milky sera without atherosclerosis In an alloxanized animal with 2000 mg percent of cholesterol and a milky serum no atherosclerosis develops because the right molecules are not present They are up in the 40 to 100 S_f class instead of down in the 10 to 30 S_f class

Katz I would not argue about that We are not at all in agreement with the Moreton concept of the role of macrochylomicronemia in atherogenesis However let me caution that the case for the S_f 10 30 molecules is still only a circumstantial one

The ovulating female chick exhibits essentially the same cyclic plasma pattern of hyperlipemia and hypercalcemia as the pigeon This is a hyperphospholipemic hypercholesteremic hyperlipemia with the hyperphospholipemia predominating Hence the C/P ratio is depressed Zondek Riddle Lindsay Marx and others showed quite a few years ago that estrogens are responsible for these plasma changes This finding led Lindsay Chaikoff *et al* as well as ourselves to give cockerels estrogens chronically As already indicated hyperlipemia and atherosclerosis result in these male chicks To our

knowledge careful studies of atherosclerosis are not yet available on mature hens undergoing such periodic physiologic hyperlipemic episodes Almost certainly the diffuse lipid infiltration of the thoracic aorta in such hens (described by Dauber) is secondary to this hyperlipemia since estrogen treated cockerels exhibit the same phenomenon Further work on this problem is currently in progress in our laboratory under Dr Rodbard s direction

We also investigated the question of malnourishment Our interest was of course aroused by the considerable data from man indicating a possible correlation between malnourishment and absence of atherosclerosis and between obesity and increased atherogenesis In the early experiments with Dr Dauber one of the things that worried us was that the birds on cholesterol plus 20 percent cotton seed oil looked sickly their feathers were oily and dirty their growth and development were retarded and their combs dragged Since these birds were malnourished Dr Dauber and I studied a set of pair fed birds having the same low feed intake but devoid of cholesterol No atherosclerosis supervened Cholesterol induced atherosclerosis could not be attributed to undernourishment (Subsequently Stamler *et al* showed that this nutritional disturbance was due to the toxic effects of 20 percent cottonseed oil With cholesterol diets containing 5 percent oil birds are quite normal We have therefore abandoned use of 20 percent oil) More recently Rodbard *et al* in our laboratory have quantitated the effects of malnutrition on cholesterol induced atherosclerosis He attacked the problem Is undernutrition protective against atherosclerosis because of overall low caloric intake or rather because of low intake of a specific atherogenic stimulus e g cholesterol In one study he compared hypercholesteremia and atherosclerosis in chicks on *ad lib* cholesterol diets vs birds eating 60 percent as much of the same diets The underfed chicks consuming only 60 percent as much cholesterol as the controls nonetheless had as marked or even more marked hypercholesteremia and atherosclerosis Thus undernutrition is not protective against hypercholesteremia and atherosclerosis in the presence of the specific atherogenic stimulus cholesterol

A second experiment on this problem is illustrated in Table XV Group 1 had 2 percent cholesterol *ad lib* Group 2 had 2 percent cholesterol alternating every 1 5 days with plain mash Group 3 had 2 percent cholesterol alternating every 1 5 days with starvation Note that when 2 percent cholesterol mash was alternated with plain mash (Group 2) the cholesterol level in the blood was

TABLE XV

Effects of Intermittent Starvation on Cholesteremia and Atherogenesis in Cholesterol Fed Cockerels

	Plasma Total Cholesterol mg %	% with Thoracic Aorta Lesions	% with Lesions in Whole Aorta	% with Thoracic Lesions Grade 1 or >	% with Whole Aorta Lesions Grade 1 or >	Mean Gross Grading — Birds with Lesions	
						Thor	Whole Aorta
Group 1 2 C Ad Lib	334	73	100	55	91	1 8	2 9
Group 2 2 C Alternate with Plain Mash	190	33	92	0	42	0 4	1 1
Group 3 2 C Alternate with Starvation	483	100	100	100	100	2 8	3 6
Plain Mash	88	0	60	0	0	0	0 5

Data obtained after 20 weeks of the experimental regimens *Reprinted from* Katz and Stamler *Experimental Atherosclerosis* Charles C Thomas Springfield Ill in press

significantly reduced compared with Group 1 However when it was alternated with no food (Group 3) the hypercholesteremia was more severe than in chicks receiving cholesterol continually

Gofman May I ask what the range was in group 3 in those alternated with starvation? What was the lowest and highest?

Stamler The ranges were Group 1 — 164-379 mg percent Group 2 — 169 215 Group 3 — 306 948 The differences between the means for Group 2 vs Groups 1 and 3 are statistically significant

Kellner Are these cholesterol figures the means of the highest levels obtained or the averages of all the levels for the birds for the duration of the experiment?

Stamler These are the data at 20 weeks The findings were consistent throughout the experiment The chicks were bled at 1 3 5 10 15 and 20 weeks these 20 week data are in agreement with what was recorded throughout the experiment

Katz In the plain mash group (Group 4) there were no thoracic lesions In the 2 percent cholesterol group 73 percent had thoracic lesions With cholesterol plain mash alternating (Group 2) — and this is the significant thing — 33 percent had lesions much less than Group 3 (cholesterol starvation alternation) where 100 percent of birds had lesions (Table XV) In other words if the chicken eats plain mash alternately the cholesterol tends to disappear to be demobilized from the blood vessel as well as from the blood and when starvation intervenes it is not so demobilized — as a matter of fact it is mobilized more and the lesions are greater One may suggest that excessive cholesterol is or is not mobilized and disposed of depending on diet influenced metabolic factors

Shorr Do you care to say anything more as to the mechanism involved? Do the birds undergo any change in over all metabolism or mobilize their fats and so on?

Katz That is still in the speculative stage It may well be related to the over all metabolic and endocrine pattern in the starved vs the plain mash alternating chicks These chicken experiments would seem to parallel Malmros observation that in Denmark during the war when the normal diet was restricted but high in cholesterol there was no fall in death rate from arteriosclerosis In contrast in Norway when the restricted diet was low in animal fats and cholesterol fewer arteriosclerotic deaths occurred In starvation and in

restricted diet, the presence or absence of specific atherogenic substances is the determining factor Starvations are not all of the same kind There probably are also different types of overnutrition

Shorr I take it that Group 3 was not fed thyroid

Katz We did not feed thyroid to Group 3

Stamler These semistarved birds are obviously hypoendocrine We recognize the possibility that the different results in the two alternating groups (starvation vs plain mash) may be related to depression of thyroid function occurring with undernutrition

Shorr Also the gonads should be looked at

Goldblatt Have you had a look at other organs?

Stamler The testes are small The comb indices are low There is obviously diffuse hypoendocrinism But depression of which endocrinism is responsible for this?

Kellner Do they not catch up on the days when they are fed?

Stamler Not usually A chick eats almost continually all day long It is very difficult for it to catch up on the alternate day

Gofman Do they lose weight?

Stamler Yes they are smaller

Kellner What happens to the cholesterol level of a chick that is starved on a plain mash diet?

Katz We have not studied that It has been suggested that a low fat diet may be prophylactically or therapeutically valuable for human atherosclerosis Horlick and I therefore undertook to study spontaneous atherogenesis in cockerels after 63 weeks on a defatted mash diet containing about 0 3 to 0 4 percent total fat and no cholesterol We compared them to birds fed ordinary mash which contains 3 5 percent total fat and about 300 mg percent cholesterol The birds fed a fat free mash for 63 weeks still had spontaneous lesions of the abdominal aorta However these lesions were delayed in onset occurred less frequently and were less severe These differences were suggestive but not conclusive in view of the small size of the experimental group Interpretation of these findings in relation to man is difficult particularly in view of our lack of clear understanding of the pathogenesis of the spontaneous lesion which may well be primarily a fibrotic rather than an atheromatotic lesion

In chicks implanted with stilbestrol the fat free diet had little effect on either the endogenous endocrine induced chronic hyperlipemia or on the resultant atherosclerotic lesions in the thoracic aorta

Shorr Would you refresh me as to whether or not the serum calcium rises?

Stamler It does go up

Shorr You therefore have another factor to bear in mind namely hypercalcemia I was thinking of this in connection with your statement that you could not as effectively alter the pattern resulting from stilbestrol as you could the pattern due to cholesterol feeding

Katz Our findings are reversibility of cholesterol induced atherosclerosis in cockerels are summarized in Table XVI On the cessation of cholesterol feeding after 10 weeks on a 2 percent cholesterol diet hypercholesteremia disappears within a few days Over the ensuing weeks lesions regress and may even be completely resorbed In contrast continuation of cholesterol feeding results in progressively more severe lesions Hence in chicks as well as in rabbits (as Anitschkow showed many years ago) and presumably in man(2) lesions can be reversed Some of the chickens have no lesions 14 weeks after cholesterol withdrawal In others where apparently the lesions are more severe the cholesterol and lipid tend to disappear

TABLE XVI

Retrogression of Atherosclerotic Lesions on Cessation of Cholesterol Feeding in the Chick

	Number of Chicks	Average Gross Grading of Lesions
Controls Cholesterol Fed for 10 weeks	18	2 5
Controls Cholesterol Fed for 15 24 weeks	19	4 6
Experimental Cholesterol Fed for 10 weeks then plain mash for 5 14 weeks	11	2 0
Experimental Cholesterol Fed for 10 weeks then low fat mash for 5-14 weeks	16	1 2

Reprinted from Katz and Stamler *Experimental Atherosclerosis* Charles C Thomas Springfield Ill in press

but fibrosis and calcification proceed and one finds regression rather than disappearance

Ogden When you state that this can be reversed similarly in the rabbit and presumably in man these data would be referable to children or youths rather than to the middle aged individuals who ordinarily have the disease *In other words you are using a different age group in the chicken*

Katz That is fair They would be about fifty weeks old at the most, and that is about one twentieth of their life expectancy

Ogden Have you done this sort of thing in elderly chickens?

Katz We have no data on reversibility of cholesterol induced *lesions in elderly birds*

Stamler I think we should defer to Dr Wilens on the subject of reversibility in mature human beings

Wilens Our studies(2) on necropsy material have indicated that if a person loses considerable weight during a period of 2 to 3 months before death the aortic plaques are likely to contain less lipid than if such a period of terminal wasting had not occurred It is suggested therefore that in some instances resorption of arterial lipid deposits may be associated with general wasting of tissues

Sims Did that include the cholesterol crystals?

Wilens The most striking change was a diminution in the number of foam cells but the total amount of fat often appeared to be reduced

Katz We have completed some experiments with the lipotropic factors choline and inositol in which we found them to have no prophylactic effect on blood cholesterol levels or on severity of lesions in either spontaneous or cholesterol induced or stilbestrol induced atherosclerosis These completely negative findings are in agreement with those of Firstbrook on rabbits and Davidson *et al* on dogs Gofman also reported on the lack of effect of the lipotropic factors *on* plasma lipoprotein levels in man We therefore stress that there is no basis of research data for claims that these factors are useful in human atherosclerosis The few claims of positive results in man are uncontrolled clinical observations Dr Rodbard has recently undertaken another study on chickens of the effects on cholesteremia and atherogenesis of an aluminum hydroxide gel

which adsorbs cholesterol *in vitro* It was suggested that feeding this material might alleviate the effects of ingested cholesterol The results which are still not in final form suggest that in cholesterol fed chicks the aluminum hydroxide gel depresses hypercholesteremia and atherogenesis However no effect was observed on silbestrol induced lesions and the associated endogenous hyperlipemia We are currently testing this preparation on man

Shorr One thing to bear in mind Dr Katz is that aluminum hydroxide gels have an even greater binding capacity for phosphates The result in animals that are consuming aluminum phosphates all the time will be to reduce the level of phosphate ion in the blood This reduction introduces another variable which may have to be evaluated

Katz It may not act on cholesterol but on some other substance involved in atherogenesis

Goldblatt Would you care to mention the nature of the lipotropic substance that you used?

Katz In our studies with lipotropic factors we used 1 percent choline + inositol mixed in with the diet No lipotropic factors were used in the aluminum hydroxide experiments

Dock What percentage of aluminum hydroxide is in the mash?

Stamler Four to 18 percent in different experiments

Katz Dr Dauber Dr Horlick and Dr Stamler have carried out various studies with thyroid hormone First we showed that in cholesterol fed cockerels thyroid powder mixed in mash depressed hypercholesteremia and lessened the severity of lesions No such definitive effects were obtained with potassium iodide in intact chicks Next it was demonstrated that thyroid also inhibited stilbestrol induced atherogenesis although depression of hyperlipemia was observed only during the initial weeks of the experiment One of the things that concerned us was Does thyroid act by virtue of its overall general effect on metabolism or by some more specific mechanisms? Stamler compared the effects of dinitrophenol and thyroid both of which increase metabolism In contrast to thyroid dinitrophenol failed to depress either hypercholesteremia or atherogenesis in cholesterol fed cockerels The thyroid powder apparently exerts its effect by some other mechanism than its basal metabolic stimulating action At present we are comparing the

effects of thyroid stimulating hormone of the anterior pituitary (TSH) and desiccated thyroid

Shorr It is hazardous to regard the stimulation with dinitro phenol as being comparable to that obtained with thyroid extract Actually it is quite definite that the effects on metabolism are achieved through different mechanisms

Katz That is true The problem of atherosclerosis in diabetic man led us to study depancreatized chickens Dr Stamler found that in depancreatized birds fed 2 percent cholesterol — 5 percent cottonseed oil mash the resultant hypercholesteremia and athero genesis are inordinately severe The depancreatized chick on regular mash exhibits neither hyperglycemia nor hyperlipemia No diabetes develops Stamler and Pick therefore undertook to produce diabetic birds for the study of atherogenesis They found that adrenal cortical extract (ACE) produces a marked hyperglycemia in depancreatized cockerels In cooperation with R Levine in the Department of Endocrinology they are studying this problem fur ther They have found that neither ACTH nor growth hormone nor cortisone nor DCA produce this hyperglycemia in pancre atectomized chicks Adrenal steroid compound F does but the hyperglycemia is less severe than with ACE

It is of interest that the inordinate hypercholesteremia exhibited by depancreatized chicks requires a supplement of both cholesterol and cottonseed oil in the mash with cholesterol alone for unknown reasons it does not occur This finding served suddenly to reintro duce the oil or neutral fat factor into our thinking

Perera Is this total or subtotal pancreatectomy?

Stamler If ones surgical procedure is efficient in about 80 percent of the birds total pancreatectomy is achieved in so far as one can tell histologically by section of the loop of gut in which the pancreas lay In about 5 10 or 20 percent of birds not quite all of it is removed

Dexter Is there any reason to believe that this total or subtotal pancreatectomy is in any way enhancing absorption of cholesterol in the gut or do you think it is working through its endocrine function in producing this hypercholesterolemia?

Katz Stamler has an experiment presently in progress which attempts to get at the mechanism involved

We have been able to produce moderate hypertension in chickens by salt feeding Dr Lenel spent over a year trying to produce it by ligation of the ureter by a clamp on the renal artery, by a cast or cellophane around the kidney etc The chicken does not lend itself to those procedures Later Stamler *et al* showed that with considerably smaller amounts of salt plus desoxycorticosterone acetate moderate hypertension also supervenes The effects of these hypertensions on chick atherogenesis may be briefly summarized In chicks fed plain mash (without a cholesterol supplement) hypertension does not cause the appearance of thoracic aorta lesions of the induced type nor does it intensify spontaneous arteriosclerosis When cholesterol is added to the mash hypertension (caused by DCA + salt) does have a demonstrable intensifying effect upon the cholesterol induced atherosclerosis

Gofman What were the cholesterol levels of the two groups?

Katz The cholesterol levels in the two cholesterol fed groups with and without DCA + salt were almost identical

To summarize our conclusions from these experiments Hypertension (due to DCA + salt) intensifies atherogenesis only in the presence of the prerequisite atherogenic stimulus i e an altered lipid cholesterol metabolic pattern Another problem that we have attacked experimentally is the relationship of chick age to atherogenesis Rodbard *et al* investigated this problem by placing cockerels on a 2 percent cholesterol mash beginning with the first day of life Several facts emerged from this study First cholesterol induced gross lesions of the thoracic aorta were seen in birds as young as 5 weeks of age We have no reason to believe that these baby chicks had been subjected to any infection to any trauma to anything more than the cholesterol in their diet Certainly no senescent changes had as yet occurred in these juvenile arterial tissues We interpret these findings to mean that with the prerequisite alterations in lipid cholesterol metabolic patterns atherogenesis proceeds — even in the absence of arterial injury or senescence

Second we found that with a given fixed cholesterol diet the degree of hypercholesteremia varied with age At about 8 weeks of age the cholesterol level rose sharply Prior to this time little atherogenesis occurred thereafter it proceeded rapidly This is not a cumulative effect resulting from continuous cholesterol feeding since the same phenomenon was observed in birds put on this 2

percent cholesterol diet at 4, 6 and 8 weeks of age Apparently endogenous factors varying at different ages control the cholesteremic and atherogenic responses to exogenous cholesterol Numerous data indicate a similar phenomenon in man

The mechanism of this phenomenon in chicks remains to be elucidated It may be related to their sexual development — at 10 weeks the masculinity of these birds develops rapidly, as indicated by comb and testes growth Hence Rodbard is currently investigating the possible influence of gonadal hormones on the cholesteremic and atherogenic responses to a cholesterol diet Other endocrines may be involved e g the thyroid

To summarize the current status of our work the foregoing studies — plus our investigations on the effects of pancreatectomy steroids thyroid sex and species differences etc — all have led us in the direction of studying the endogenous factors which control lipid cholesterol metabolism Along these lines of research we feel will be found significant answers which will fill in necessary details of our knowledge of *atherosclerosis* — details which will eventually indicate the path to be pursued if the atherosclerotic lesion is to be eliminated as a pathologic, morbidity and mortality producing entity

Dexter Granted that cholesterol feeding produces atherosclerosis in animals and has proved to be very effective experimentally and that conditions associated with hypercholesteremia in man are associated with a higher incidence of atherosclerosis is it warranted to imply that cholesterol is the only or the major factor involved in human atherosclerosis?

Katz That is a good question I would divide it in two parts If *the question is* Is that the only factor? I would say No I think it is hazardous to pin all one s hope on one factor If the question is Is cholesterol the major factor? I would say In the *present state* of our knowledge subject to change without notice yes

Gofman Are you referring to cholesterol as an ingested chemical or to its blood level?

Katz I was not referring to cholesterol in the food I was referring to both *exogenous and endogenous cholesterol* As I have already indicated we put forward a general approach to the atherosclerosis problem which we call the cholesterol concept of

atherogenesis" Its essence is simply The key factor in atherogenesis is altered cholesterol metabolism Present knowledge does not permit a more specific thesis in our opinion The term "altered cholesterol metabolism" encompasses both exogenous and endogenous cholesterol the hormonal metabolic processes controlling cholesterol metabolism cholesterol *per se* and the lipids and proteins involved in its biologic absorption turnover synthesis degradation transport and excretion Our general concept serves only as an overall frame of reference guiding specific research efforts

Shorr I wonder whether any consideration has been given to the possibility that spontaneous atherosclerosis followed by spontaneous regeneration might occur in birds like the pigeon and the dove The studies of Riddle(3) and others on these two birds have disclosed a characteristic cycle involving both blood lipids and calcium with its peak just prior to ovulation The rises which occur in both calcium and plasma lipids are very great at this time The serum calcium may rise to 55 mg percent from approximately 9 mg percent and the serum lipids attain levels of over 3 000 mg percent in the pigeon These high values which extend over a period of about 100 or more hours are then followed by a return to normal values which persist for a number of months before the next ovulation This is an interesting phenomenon to study in relation to the possibility that alterations in the blood vessels of a reversible sort might occur during these hyperlipemic and hypercalcemic episodes

Stamler The effort to produce atherosclerosis with stilbestrol resulted precisely from those observations A decade or two ago several groups demonstrated that the hypercholesteremia hyperlipemia and hypercalcemia in egg laying birds were due to estrogenic hormones Based on these facts Lindsay Chaikoff and associates administered estrogens chronically to cockerels Hyperlipemia developed and atherosclerosis eventually supervened Hens maintained on normal diets exhibit an incidence of spontaneous lesions in the abdominal aorta similar to cockerels At least this is what present data indicate However these data are by no means extensive Information on wild fowl collected by Fox indicates a significant sex *difference* with the male being more prone to spontaneous lesions than the female

Further another type of "lesion" occurs in hens At autopsy they exhibit a diffuse gross yellowing of the thoracic aorta without elevation or thickening Microscopically no foam cell cushioning or fibroblastic proliferation are observed there is only a diffuse

lipid infiltration This was described by both Fox and Dauber before the stilbestrol work had been done, at that time this finding was called the second spontaneous lesion of the female chick occurring *in the thoracic aorta Its pathogenesis* was *unexplained* by these original workers I think that today based on the experimental work with stilbestrol in males, wherein the same lesion is reproduced, one may conclude that it is secondary to stilbestrol hyperlipemia This is a diffuse lipid infiltration of the arch of the aorta It is certainly not a thickening and narrowing of the vessel, and in that sense it is not atherosclerosis That is the current status of knowledge at present Dr Rodbard is studying spontaneous and cholesterol induced atherosclerosis in male vs female chicks

Shorr My reason for suggesting the pigeon instead of the hen was that the hyperlipemia and hypercalcemia are extreme and do last for almost 100 hours and then there follows a long period over which the values for both are entirely normal

Stamler I might just add one thing We have studied a few immature ducks male and female They exhibited no essential sex difference in response to cholesterol feeding

Helmer Do you have any evidence that in these chicks you can produce coronary insufficiency either functionally or anatomically, or insufficiency in any other organ functionally or anatomically, with these lesions?

Katz Cholesterol fed chicks develop lesions of the coronary arteries We are also studying the small vessels of other organs We have not observed organ changes which could be attributed to ischemia or infarction secondary to atherosclerosis We have seen no gross or microscopic evidence of myocardial infarction ECGs were not unusual in a few birds we studied with gross coronary atherosclerosis

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EFFECT OF EXPERIMENTAL RENAL HYPERTENSION ON EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS*

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THE ROLE of hypertension in the pathogenesis of atherosclerosis in man is controversial. There is some clinical evidence that hypertension both accelerates the onset and accentuates the progress of atherosclerosis. On the other hand, pathologists are familiar with instances where a patient comes to necropsy with extensive lesions of atherosclerosis and a history of normotension. There are experiments in animals, however, indicating that increased blood pressure accelerates the onset and accentuates the progress of spontaneous atherosclerosis, including those of Dill and Isenhour(1) and of Wilens(2) on the rabbit. During the progress of our work, Trianta-filo Lira and Mardones(3) reported that rabbits with experimental renal hypertension developed more severe and extensive lesions of experimental cholesterol atherosclerosis than did normotensive control animals. We also have the recent evidence which Dr. Katz and co-workers reported for the chicken. However, experimental cholesterol atherosclerosis in the rabbit differs importantly from human atherosclerosis, particularly in anatomical distribution of the gross lesions. And there is, of course, a considerable phylogenetic difference between the chicken and man. Accordingly, it seemed desirable to study the interrelationship of hypertension and atherosclerosis in another species. The work of Kendall and associates(4) showing that experimental cholesterol atherosclerosis similar in anatomical distribution and other characteristics to human atherosclerosis can be produced in the dog by thiouracil-cholesterol feeding, and the classic method of Goldblatt for producing experimental renal hypertension in this species afforded an obvious combination for studying this problem. Our results to date are incomplete but sufficiently definite to warrant a progress report.

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† Mrs. Gloria Fein and Mr. Frank Kajikawa rendered all-important technical assistance in this research.

METHODS

Hypertension was produced in 11 dogs by bilateral renal artery constriction with an intervening three weeks interval One week to one month after the second renal artery constriction, the dogs were given orally 0 1 gm kg of thiouracil and 1 0 gm kg of cholesterol daily Eleven control normotensive dogs were similarly treated except that they were subjected to sham renal operations Direct mean femoral arterial blood pressure readings were obtained semiweekly, serum cholesterol and lipid phosphorus were determined biweekly clinical examinations and weighings were made monthly or oftener when indicated, urinalyses were carried out bimonthly or more often, blood urea nitrogen determinations were made when indicated and a limited number of funduscopic examinations and a few electrocardiograms were recorded

At monthly intervals from 3 to 7 months and after 10 months of thiouracil cholesterol feeding one or more pairs of matched renal hypertensive and normotensive dogs were sacrificed and a thorough necropsy performed Gross atherosclerotic lesions were graded from 0 (no lesions) to 5 (numerous severe and ulcerative lesions) and tissues were taken for microscopic study

RESULTS AND DISCUSSION

Table XVII summarizes our results to date The gross lesions in 11 renal hypertensive dogs averaged 3 0 as compared with 0 5 in the normotensive controls Some 450 renal hypertensive dogs necropsied after 3 to 84 months of hypertension without thiouracil cholesterol feeding showed no gross atherosclerotic lesions and were graded 0 We and many others of course have observed no gross atherosclerotic lesions in many normotensive dogs of varying ages necropsied over a period of years

Table XVIII further indicates that renal hypertension accelerates the onset of cholesterol atherosclerosis and accentuates its progress The normotensive animal graded 4 had the highest pressure of this group (average mean femoral pressure 140 mm Hg)

From Table XVIII it is apparent that our results with normotensive dogs are comparable with the earlier findings reported by Kendall and his group in that we did not observe gross atherosclerotic lesions after 3 and 4 months of thiouracil cholesterol feeding More recently Kendall and associates have reported gross lesions after such a short interval(5) Perhaps the difference is

TABLE XVII

Experimental Cholesterol Atherosclerosis in Chronic Renal Hypertensive and Normotensive Dogs

No of Dogs	Description	Duration of Thiouracil cholesterol Feeding	Gross Atherosclerotic Lesions
11	Chronic renal hypertensives	3-10 months	3 0
11	Sham operated normotensives	3 10 months	0.5
450	Chronic renal hypertensives	—	0 0

TABLE XVIII

Duration of Thiouracil Cholesterol Feeding and Experimental Cholesterol Atherosclerosis

Months	Gross Lesions: Normotensive Dogs	Gross Lesions: Hypertensive Dogs
3	0 0	0 2
4	0 0 0	0 1 5
5	1 4	4 5
6	0	3
7	0 1	2 4
10	0	5

due to the fact that their most recent observations were made on puppies whereas our dogs and presumably their earlier animals were 1 to 3 years of age Also we mixed the cholesterol directly with the diet of prepared dog food (Pard) and milk whereas Kendall and associates facilitate absorption by dissolving the cholesterol in ether and allowing the ether to evaporate after mixing with the diet which they employ Nevertheless the serum cholesterol levels of our dogs are roughly comparable to those which they have reported In any event the vital necessity of a comparable control group for our hypertensive animals is emphasized

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TABLE XX
Hypercholesteremia x Time and Experimental Cholesterol Atherosclerosis

NORMOTENSIVE DOGS		HYPERTENSIVE DOGS	
Av Serum Chol mg % x Mo	Gross Lesions	Av Serum Chol mg % x Mo	Gross Lesions
1980	0	1108	0
2366	0	1150	0
2860	0	2408	1
2919	0	2610	2
3425	0	4300	5
4090	0	4650	4
4620	0	5570	2
4740	4	6010	5
5190	1	6190	3
5680	0	6770	4
5890	1	9600	5

TABLE XXI
Cholesterol Phospholipid Ratio x Time and Experimental Cholesterol Atherosclerosis

NORMOTENSIVE DOGS		HYPERTENSIVE DOGS	
Ratio x Mo	Gross Lesions	Ratio x Mo	Gross Lesions
4 4	0	4 5	0
5 8	0	5 2	0
6 5	0	5 2	2
6 7	0	5 4	1
8 5	0	8 2	4
8 6	1	8 3	5
8 8	4	8 6	5
9 5	0	10 6	3
11 3	1	10 8	4
		11 6	2

Table XXII suggests a possible correlation between the blood pressure levels and the severity of the gross lesions in both the hypertensive and normotensive dogs Obviously if such a correlation exists there are individual exceptions

Table XIX, with individual exceptions points to a correlation between the degree of the hypercholesteremia and the severity of the gross lesions in the hypertensive group Obviously the dogs showing lesions in the normotensive group are too few to permit comment from this standpoint The recorded average cholesterol level is that obtained after a fairly steady state has been reached by the end of the second month of thiouracil cholesterol feeding

TABLE XIX

Hypercholesteremia and Experimental Cholesterol Atherosclerosis

NORMOTENSIVE DOGS		HYPERTENSIVE DOGS	
Average Serum Cholesterol *mg percent*	Gross Lesions	Average Serum Cholesterol *mg percent*	Gross Lesions
710	0	554	0
817	0	575	0
922	0	964	1
1142	0	1032	4
1184	4	1114	2
1178	1	1202	5
1183	0	1240	5
1295	0	1278	3
1320	0	1305	2
1430	0	1935	4
1482	1	2150	5

Table XX is similar to Table XIX except that the duration of the hypercholesteremia (time of thiouracil cholesterol feeding) is included Again with individual exceptions there is evidence of correlation between the degree and duration of the hypercholesteremia and the severity of the gross lesions in the hypertensive group

The same may be said for the cholesterol phospholipid ratio (Table XXI) (Lipid phosphorus determinations were not made on 2 normotensive dogs and one hypertensive animal)

mm Hg) for a few animals Obviously a dog with a pressure of 130 mm. Hg following renal artery constriction is hypertensive when his normotension was 100

Grollman Still they are lower than some of your normotensive dogs

Wakerlin That is true There is an overlap between the blood pressure levels of the hypertensive and normotensive groups

Tables XXIII and XXIV suggest a correlation between the magnitude and duration of the hypertension and the severity of the cholesterol atherosclerosis Of course the correlation may actually involve pathogenetic biochemical or pathophysiological factors more fundamental than the hypertension It is to be noted from Table VII that 3 of the 11 hypertensive dogs had hyper*tensions* of only 10 mm Hg whereas the minimal hypertension in other dogs constricted by the same technique but not subjected to thiouracil cholesterol feeding is 25 mm Hg and is observed in less than one fourth of such dogs The decreased hypertensions appear to be due to thiouracil-cholesterol feeding although further work is necessary to substantiate this and to determine whether the decrease is due to thiouracil to cholesterol or to their combination Thiouracil cholesterol feeding had no significant effect on the blood pressure of the normotensive control dogs (The dog with 165 mm Hg of hypertension was a combination neurogenic (buffer nerve) and renal hypertensive animal – the only one in the group)

TABLE XXIII

Hypertension and Experimental Cholesterol Atherosclerosis

Hypertension in mm Hg	*Gross* Lesions
10	0 0 2
25	3
30	1 5
35	5
40	4
45	4
70	2
165	5

TABLE XXII

Blood Pressure and Experimental Cholesterol Atherosclerosis

NORMOTENSIVE DOGS		HYPERTENSIVE DOGS	
Mean Femoral B P mm Hg	*Gross* Lesions	Mean Femoral B P mm Hg	Gross Lesions
110	0	130	0
110	0	130	3
115	0	140	0
115	0	140	1
120	0	140	2
120	1	150	4
130	0	150	5
130	0	160	4
130	1	160	5
135	0	210	2
140	4	280	5

It is interesting to note in Table XXII that the only normo tensive dog showing grade 4 lesions had an average mean femoral pressure of 140 mm Hg which is just below what our research group *classifies as spontaneous hypertension*

Gofman Except for the last animal (with a blood pressure of 280, gross lesions of five and about twice the cholesterol level of *any of the others) there is almost no correlation with pressure per se*

Wakerlin The possible correlation shows up better with the hypertension than with the blood pressure levels as will be evident from Table XXIII This is merely a progress report and we should be able to speak with more certainty when we have doubled the number of animals in the normotensive control and hypertensive groups

Grollman How about those animals with pressures of 130 and 140 in the hypertensive column?

Wakerlin These pressures were higher than the control normo tensive levels for the same animals Normotension in our laboratory will vary from an average of 90 mm Hg (range of 80 100 mm Hg) *for some* dogs to an average of 140 mm Hg (range of 130 150

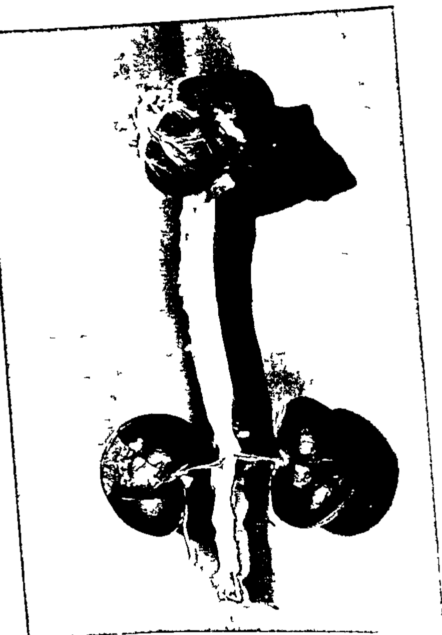


FIGURE 45 Heart opened aorta and branches and kidneys of a normotensive control dog g d d 0 aft r 10 mo thiouracil-cholesterol feeding

TABLE XXIV

Hypertension x Time and Experimental Cholesterol Atherosclerosis

Hypertension in mm Hg x Times in Mo	Gross Lesions
30	0
35	0
65	2
120	1
120	5
150	3.5
160	4
210	2
290	4
1560	5

Figure 45 shows the heart, opened aorta and main branches and attached kidneys of one of the normotensive control dogs graded 0. This is the typical picture of the normal dog with no gross atherosclerotic lesions. A normotensive control dog graded 1 showed a few atherosclerotic plaques in the abdominal aorta and in the external and internal iliacs.

Figure 46 shows the gross atherosclerotic lesions in the only normotensive control animal graded 4. Extensive ulcerated plaques can be seen in the abdominal aorta extending down into the external and internal iliacs. There are numerous plaques in the renal arteries, the superior mesenteric artery, the coeliac axis and the ureteric arteries. Unfortunately the heart is turned wrong side out for viewing the coronaries but there are extensive plaques in these arteries and their branches. You will recall that this animal had the highest pressure of the normotensive group.

Katz: What is the location of plaques in the renal arteries in relation to the Goldblatt clamps?

Wakerlin: We find atherosclerotic plaques along the course of the renal artery and its branches distal to the clamp as well as proximal to it. Two of my students, Mr. R. W. Sevy and Dr. E. W. Hawthorne, measured the mean pressure in the renal artery proximal and distal to the clamp in 11 dogs with renal hypertension of three months to seven years duration. They found the pressure averaged 40 mm Hg (20-70) lower distal to the clamp.

Shorr Was this determined by means of a mercury manometer?

Wakerlin They used an aneroid manometer checked against a mercury manometer standard

The gross lesions of the cardiovascular renal system of a hypertensive dog graded 2 are particularly evident in the abdominal aorta the superior mesenteric artery and the ureteric arteries The more severe lesions in a hypertensive dog graded 3 involve the abdominal aorta and its main branches A hypertensive dog graded 4 shows still more extensive plaque formation and ulceration in the abdominal aorta iliacs renals superior mesenteric coeliac subclavians and carotid arteries There are extensive and numerous plaques also along the ureteric and coronary arteries

Stamler Is there thrombus formation?

Wakerlin Yes there is thrombus formation in the external iliac artery in this dog

Figure 47 shows the gross atherosclerotic lesions of a renal hypertensive dog graded 5 Note the heavy plaque formation and ulceration in the abdominal aorta down into the external and internal iliacs and out into the superior mesenteric and coeliac arteries Figure 48 shows the heart of this animal with plaques along the course of the coronaries and their branches

Stamler Is there any gross evidence of fibrosis?

Wakerlin We saw no evidence of myocardial fibrosis grossly We have not examined the myocardium microscopically as yet We have done a few E K G s including one on this animal which were within normal limits All of the dogs which were graded 4 or 5 showed atheromatous plaques in their coronary arterial system similar to Figure 48 Our research group suggests that this observation may possibly be channeled into a method for the production of experimental chronic myocardial insufficiency If such an insufficiency can be produced it should resemble that seen clinically as a result of hypertensive atherosclerotic heart disease We plan to follow dogs with extensive atherosclerosis and hypertension for long periods to see if they will develop chronic myocardial insufficiency We have not thus far placed any of our dogs with atherosclerosis and hypertension on a treadmill to see if a decrease in the functional capacity of the cardiovascular respiratory systems can be demonstrated as a consequence of the degree of progressive coronary atherosclerosis now being reported



FIGURE 46 Heart opened aorta and branches and kidneys of a normotensive (mean B P 140 mm Hg) control dog graded 4 after thiouracil cholesterol feeding for 5 mo



FIGURE 48 Heart surface showing atherosclerotic lesions in coronary vessels (same dog as shown in Figure 47)

Figure 49 shows another hypertensive dog graded 5 Note that the extensive ulcerative lesions have a distribution similar to that of severe human atherosclerosis and involve the abdominal aorta iliacs renals superior mesenteric coeliac ureterics coronaries and subclavian arteries There are also a few plaques in the thoracic aorta This dog was the combination neurogenic renal hypertensive previously mentioned

We have noted extensive plaque formation in a main division of the renal artery beyond the clamp the atheromatous process extending into the renal medullary branches in a hypertensive dog graded 4 There was also in this dog a large atheromatous plaque on a branch of the mesenteric artery supplying a loop of small bowel

Plaque formation is extensive in the carotid artery and the thyroid artery of a hypertensive dog graded 5 Dr Kendall and his associates previously reported that plaque formation is frequently seen earliest in the thyroid arteries and we have found this to be the case Four of the six dogs graded 4 or 5 showed extensive plaque formation in the renal arteries both distal and proximal to the clamps In these four dogs the steady hypertensive level increased

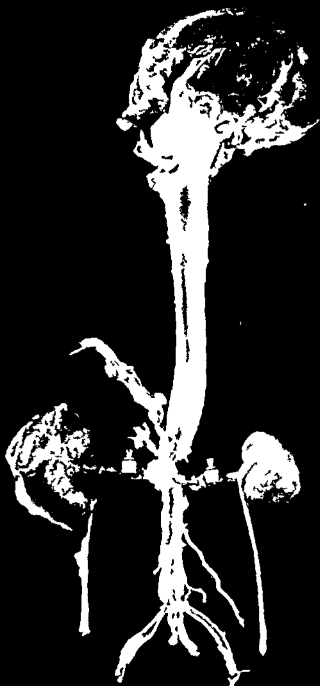


FIGURE 47 Heart opened aorta and branches and kidneys of an experimental renal hypertensive dog graded 5 after 4 mo of thiouracil cholesterol feeding

acutely one to two months prior to sacrifice in a way that rarely if ever occurs in experimental renal hypertension

Stamler Was there ever any rise in blood pressure in these dogs that might be interpreted as due to further interference with the renal circulation by an atherosclerotic plaque?

Wakerlin We are inclined to believe without definitive proof however that the accentuation of the hypertension was due to the plaque formation in the renal arteries with a resulting further constriction

Shorr Have you ever removed any of the clamps in these animals?

Wakerlin We have not tried it in any of the dogs of this experiment We have attempted to remove the Goldblatt clamp in a few renal hypertensive dogs After several months there is usually a good deal of fibrous tissue about the clamp and the arterial wall within the clamp is frequently thin and atrophic Perhaps removal of the clamp at the end of the first month of constriction would be more instructive

We have noted extensive plaque formation in the wall of the adrenal artery branch on the surface of the adrenal gland of a renal hypertensive dog graded 5 This dog also had extensive plaque formation in one of the intercostal arteries severe ulcerative lesions of the terminal abdominal aorta and the iliacs slight plaque formation in the thoracic aorta and extensive plaque formation in cortical collateral arteries supplying the kidney

The foregoing results and discussion constitute only a progress report Microscopic examination of the various tissues obtained must be carried out Additional paired renal hypertensive and normotensive dogs are under study with thiouracil cholesterol feeding Renal hypertensive dogs are also being treated with thiouracil alone and with cholesterol alone Paired neurogenic hypertensive and normotensive dogs are being studied with thiouracil cholesterol feeding and a similar study is projected for spontaneous canine hypertension Studies on monkeys with experimental renal hypertension are projected The possibility of producing experimental chronic myocardial insufficiency in dogs on an atherosclerotic hypertensive basis and the possibility of producing deficiency or alterations in function of other organs on an atherosclerotic basis will be borne in mind



Figure 49 Heart opened aorta and branches and kidneys of neurogenic renal hypertensive dog graded after 10 mo of thiouracil cholesterol feeding

Kendall As Dr Wakerlin said our animals are young They are put on experiment at the age of four months and are eight months old when sacrificed The lesions that we are obtaining resemble more the milk streaks seen in young children and infants than they do arteriosclerosis seen in adults The dogs that we have had on experiment for longer periods of time show lesions that look like those in mature human beings

Wakerlin Before we break up I should like to express the thanks and appreciation of the members of the Conference for the sponsorship of the Conference by the Josiah Macy Jr Foundation I should like to voice our appreciation to Dr Fremont Smith for his guidance to our Chairman Dr Harry Goldblatt for his competence and at times forbearance and to Miss Janet Freed for her help and to wish a long period of continued effectiveness for the Foundation

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CONCLUSIONS

1 Experimental renal hypertension appears to accelerate the onset and accentuate the progress of experimental cholesterol atherosclerosis in dogs

2 The gross lesions of experimental cholesterol atherosclerosis in dogs are similar to those of human atherosclerosis in distribution and appearance including ulceration and thrombus formation with the more severe lesions

3 The possibility of producing experimental chronic myocardial insufficiency in dogs on an atherosclerotic (coronary) hypertensive basis is suggested The possibility of producing other organ deficiencies or altered function on an atherosclerotic basis is suggested, particularly since an accentuation of experimental renal hypertension appearing to result from further narrowing of the constricted renal arteries by atheromata was observed

Gofman Dr Kendall is there a good correlation between the severity of lesions in the dog and cholesterol levels of 1000 or 1500 mg?

Kendall If you consider individual animals no but if you compare groups of three or four animals the correlation is good

Goldblatt Did you find you had to reach such high cholesterol levels as Dr Wakerlin did in order to produce the lesions?

Kendall The cholesterols were around 1000 mg

Gofman In animals with 1000 mg was the sclerosis appreciable or minimal in general?

Kendall Only in the animals that were maintained on experiment for two years did we see any lesions as severe as in many of the hypertensives Levels averaging between 2700 and 3600 mg percent were required in the normotensive animals to produce lesions as severe as those shown by Dr Wakerlin

Gofman In Dr Wakerlin's normotensive animals there is apparently no correlation between cholesterol level and atherosclerosis

Wakerlin That is true because we have not reached a point where we are producing lesions to any great extent

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